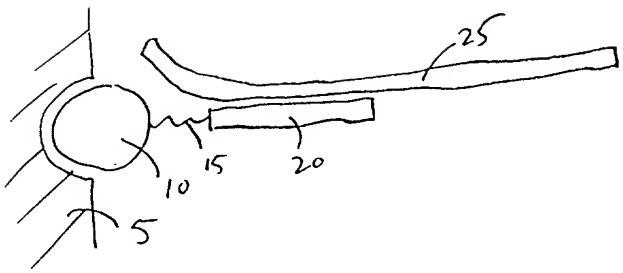
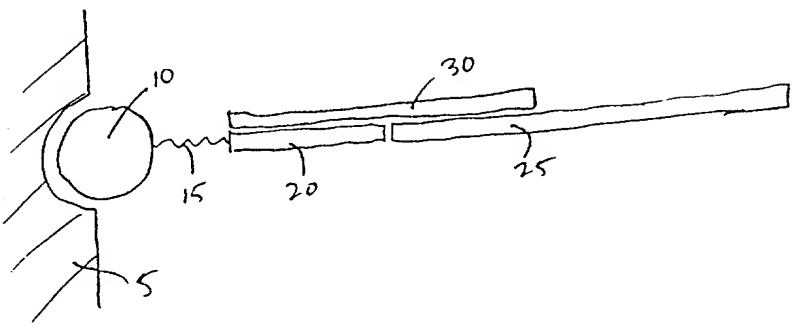
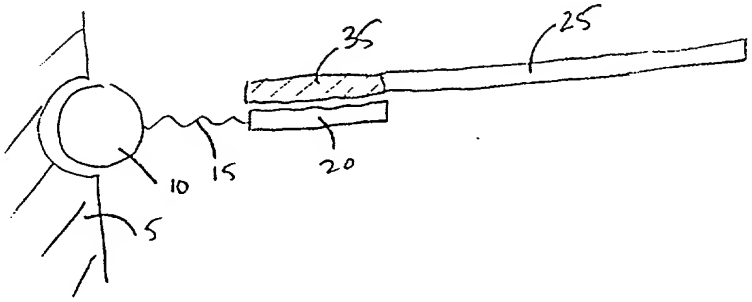


A



B



C

Fig 1

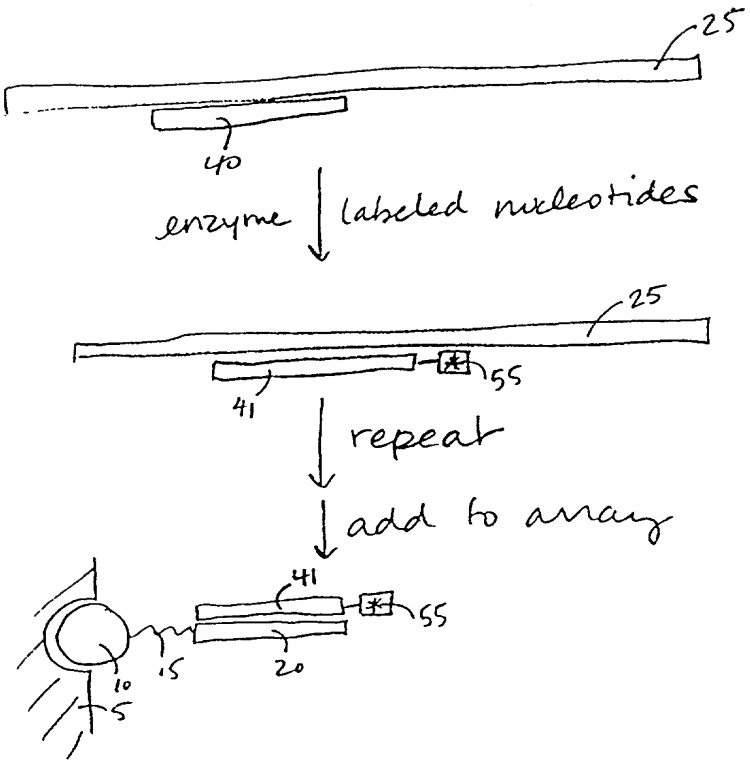

25
40
enzyme
labeled nucleotides
25
55
41
repeat
add to array
41
55
10
15
20
5

Fig 2A

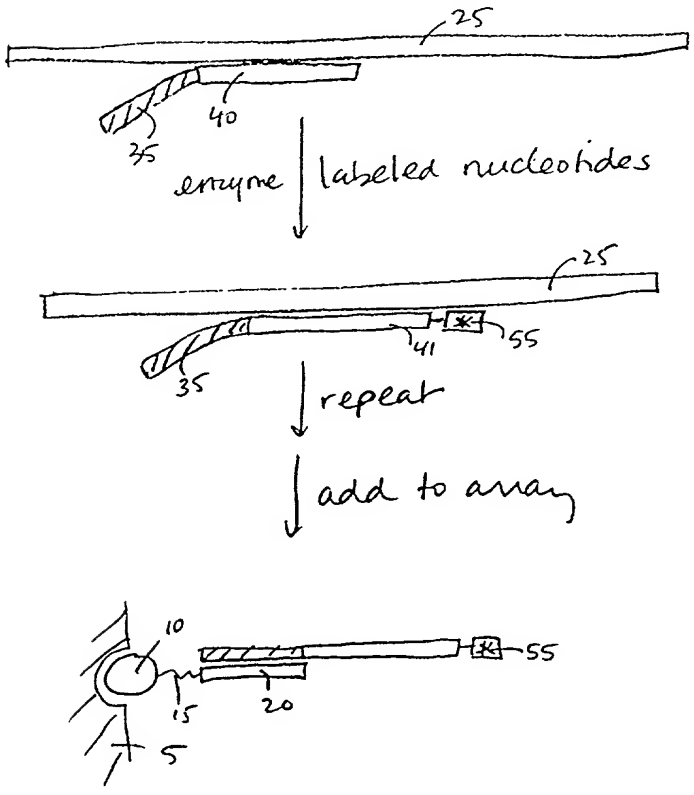

25
40
35
enzyme
labeled nucleotides
25
41
55
35
repeat
add to array
10
55
15
20
5

Fig 2B

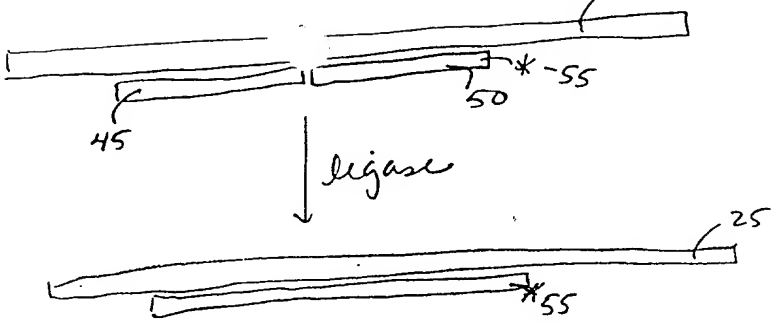
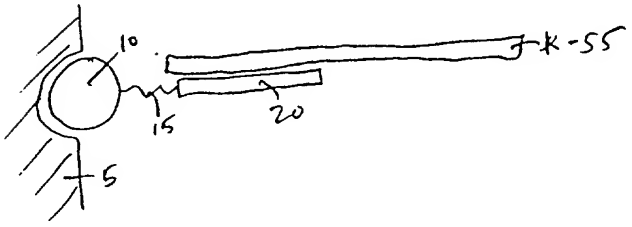

*-55
50
45
ligase
25
55

Fig 3A


repeat,
remove unligated probes as needed
10
*-55
15
20
5

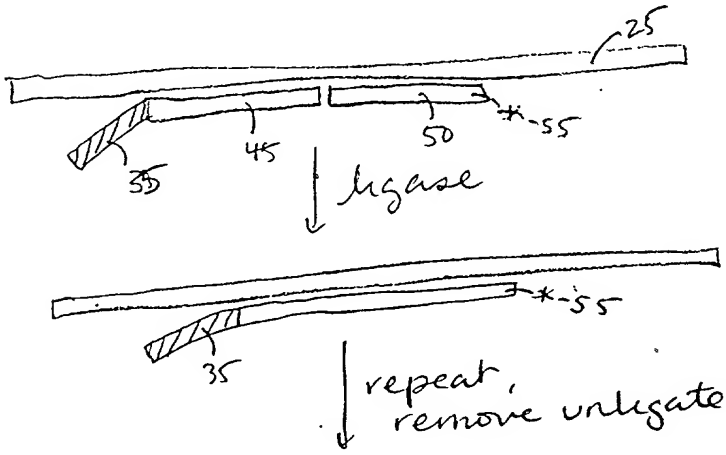
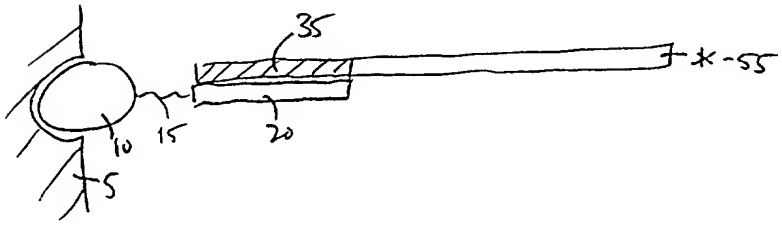

25
50
*-55
45
35
ligase
*-55
35
repeat,
remove unligated probes as needed

Fig 3B


35
*-55
10
15
20
5

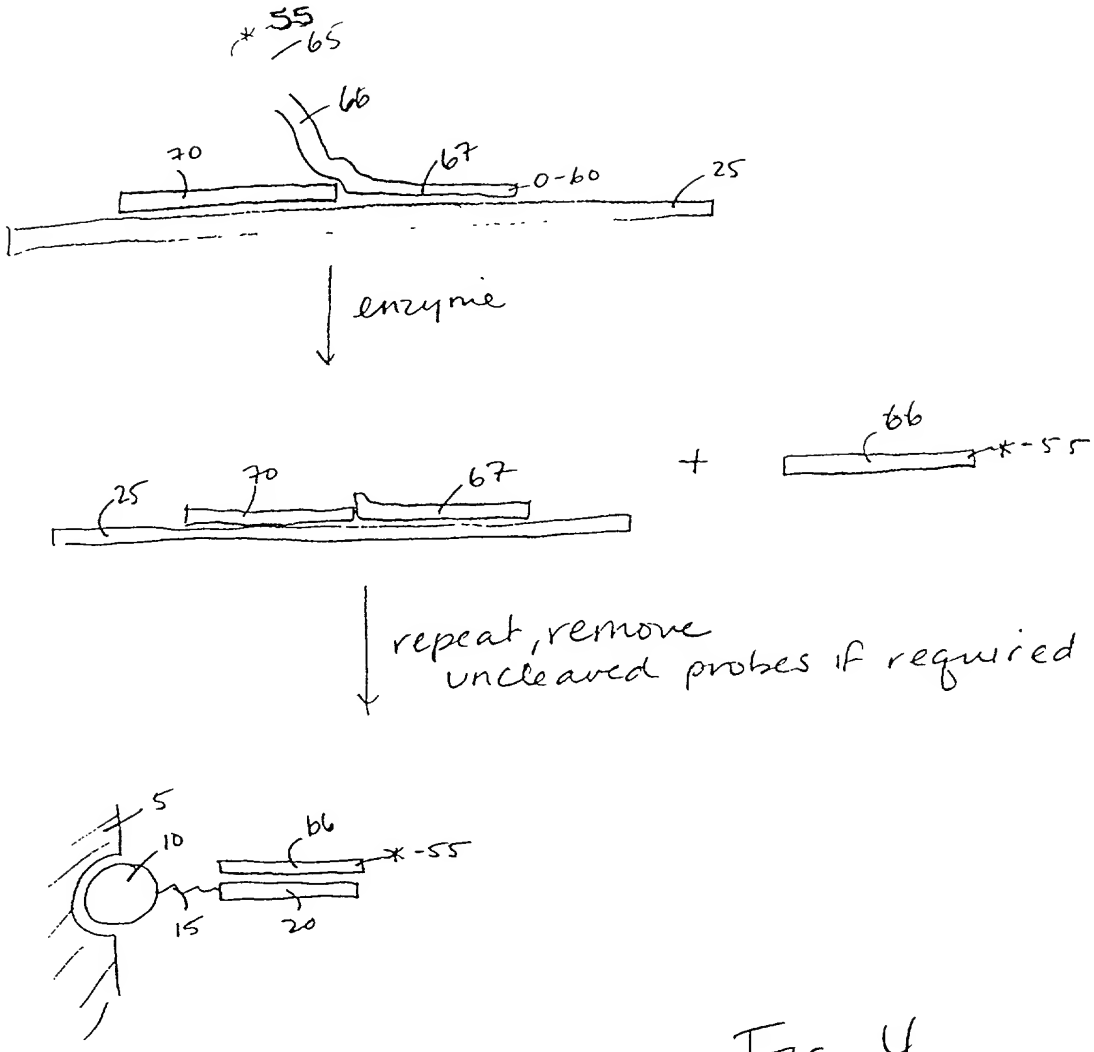

*55
65
66
70
67
60
25
enzyme
66
*-55
25
70
67
+
repeat, remove
uncleaved probes if required
5
10
66
*-55
15
20

Fig 4

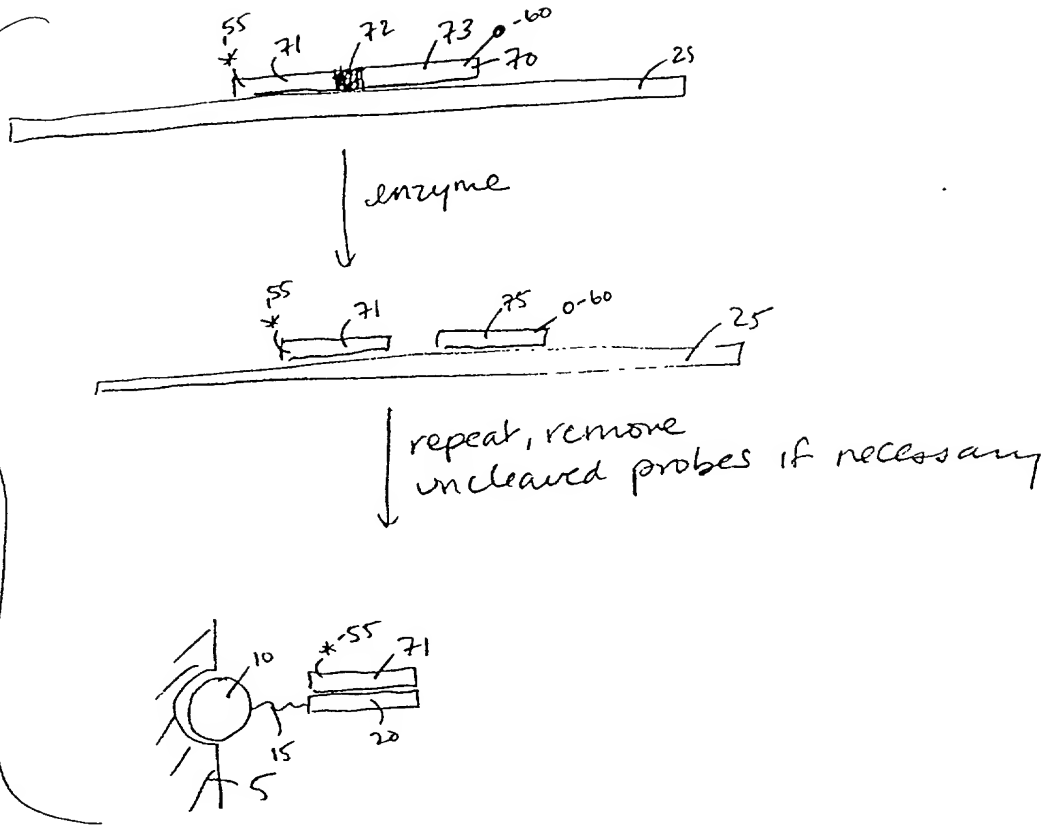

55
71
72
73
0-60
70
25
enzyme
55
71
75
0-60
25
repeat, remove uncleaved probes if necessary
10
*55
71
15
20
5

Fig 5A

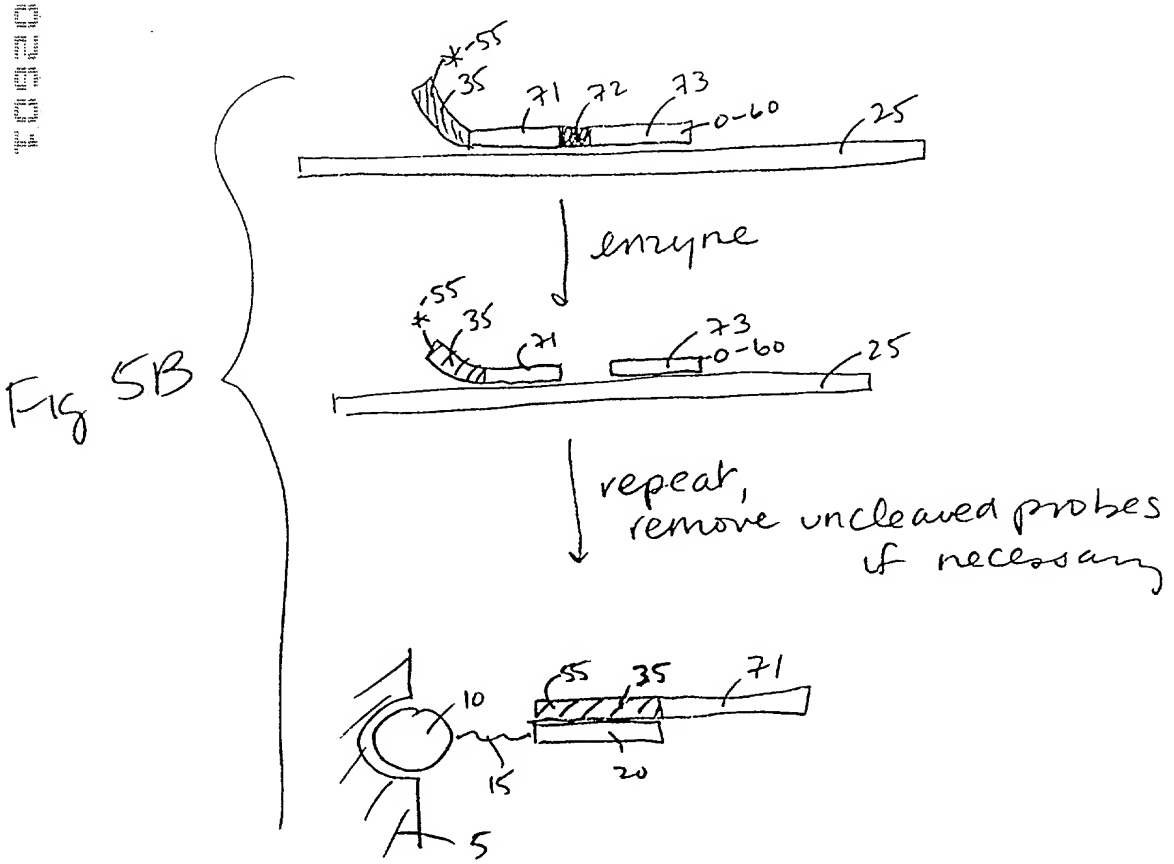

*55
35
71
72
73
0-60
25
enzyme
*55
35
71
73
0-60
25
repeat, remove uncleaved probes if necessary
10
55
35
71
15
20
5

Fig 5B

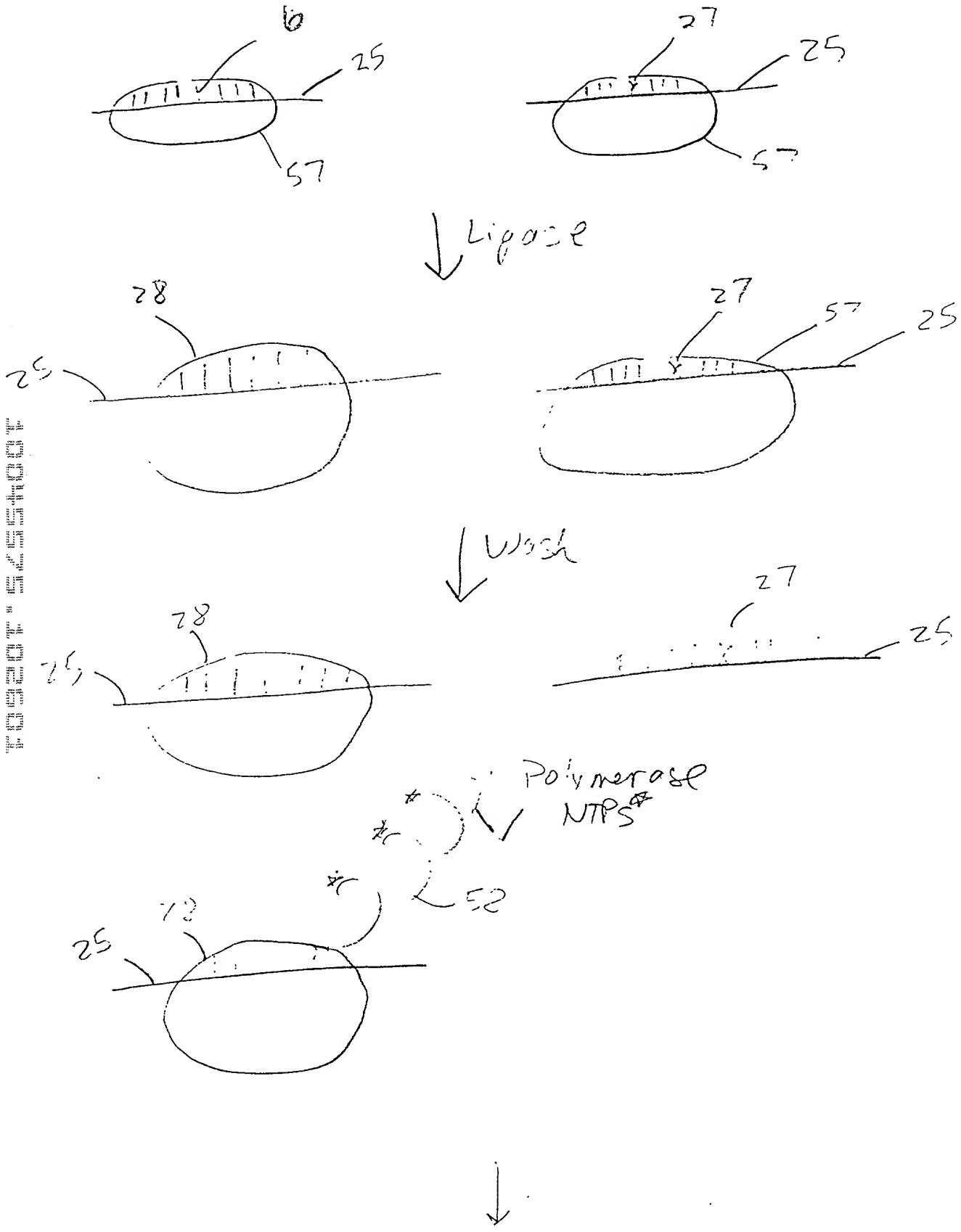

6
25
57
27
25
57
Ligase
28
25
27
57
25
Wash
28
25
27
25
Polymerase
NTPS
52
28
25

FIGURE 6

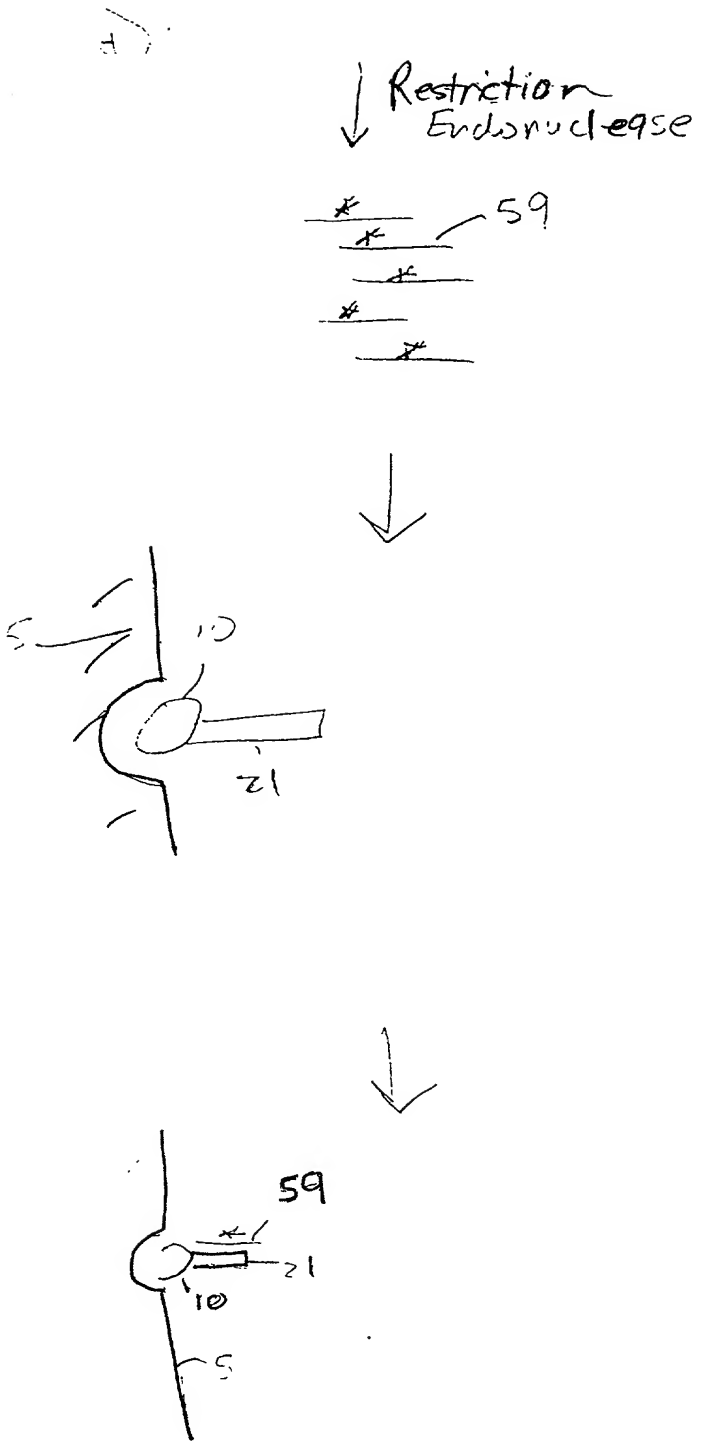


FIGURE 6
Continued

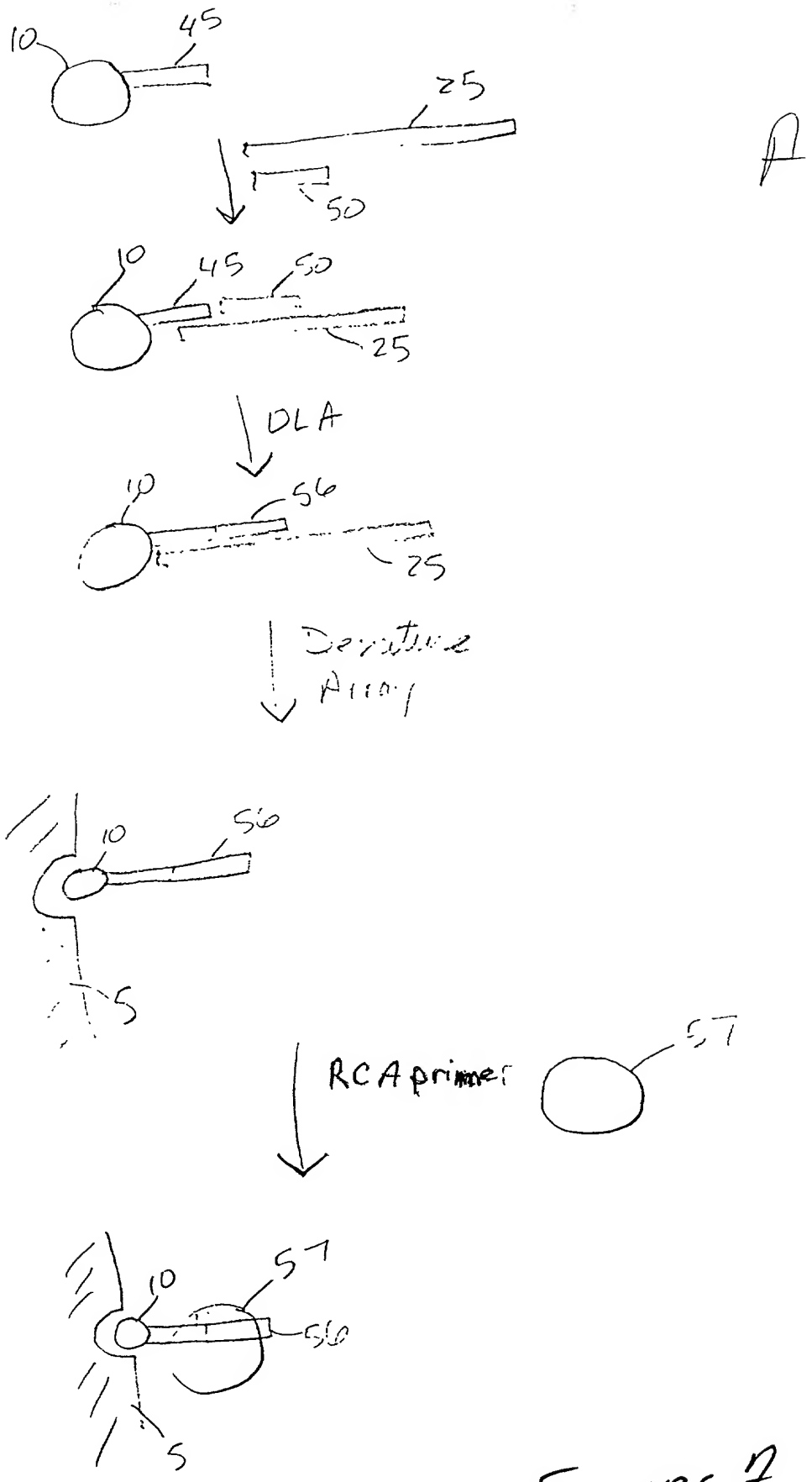

10
45
25
50
10
45
50
25
DLA
10
56
25
Derivative
Array
10
56
5
RCA primer
57
10
57
56
5

FIGURE 7

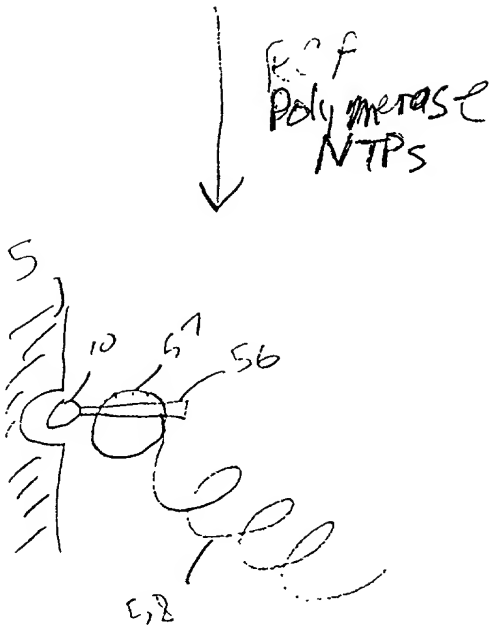
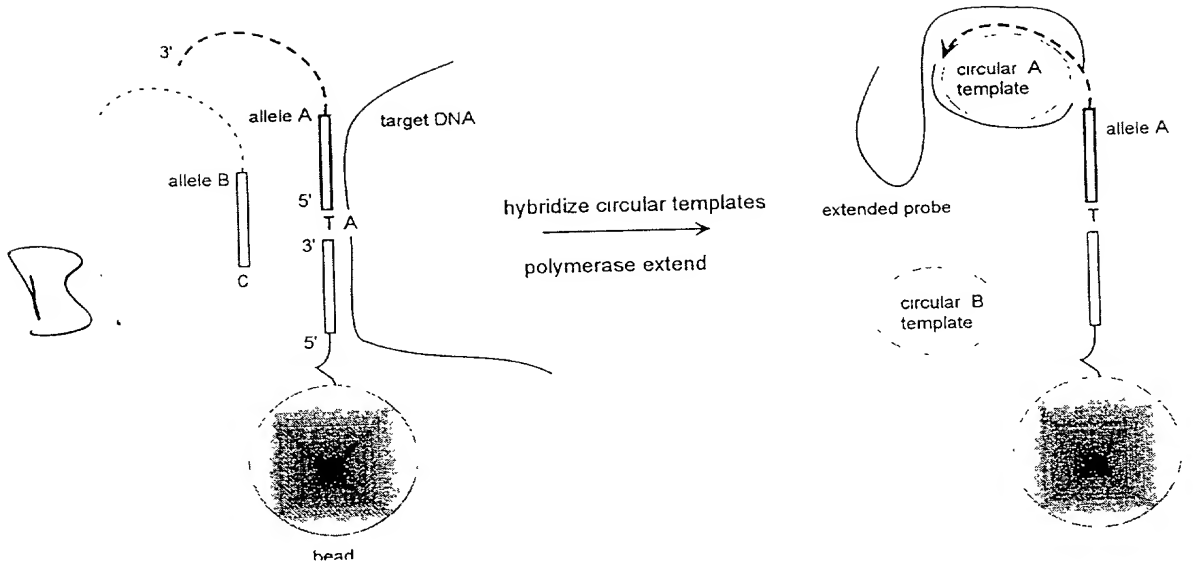


FIGURE 7
(continued)



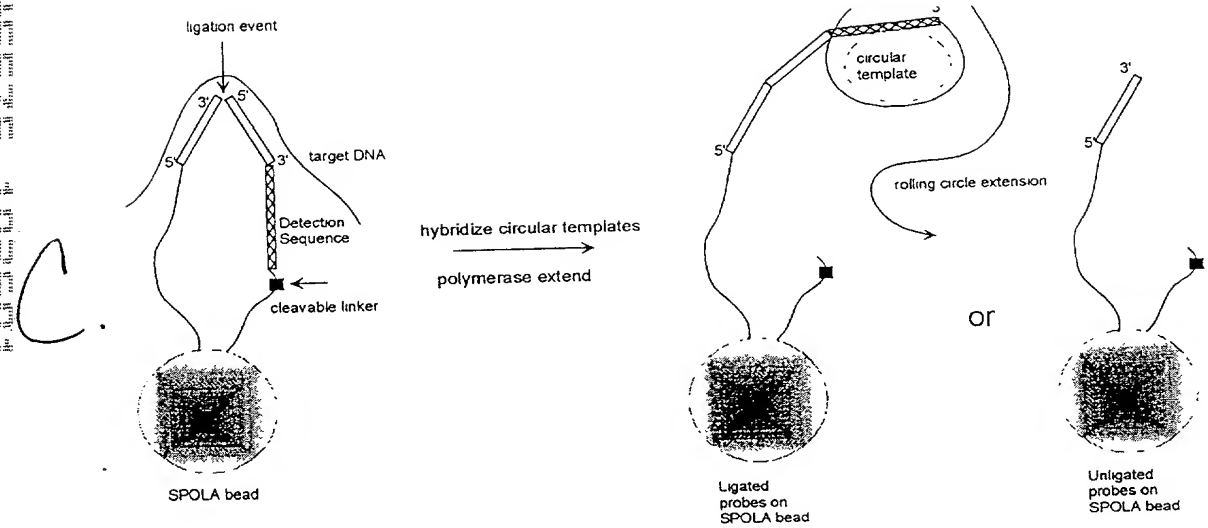
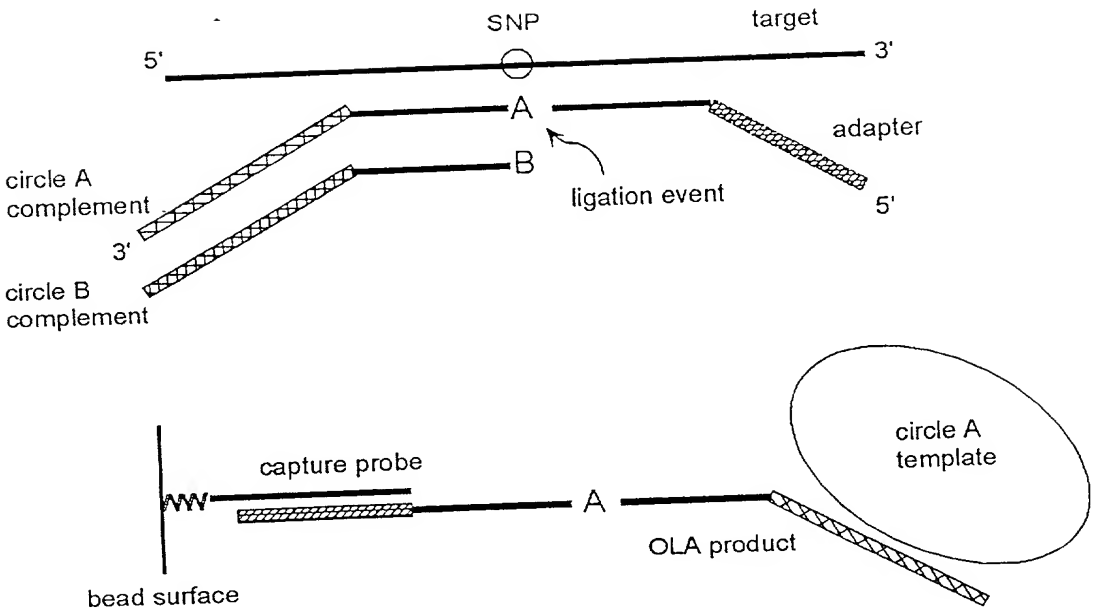
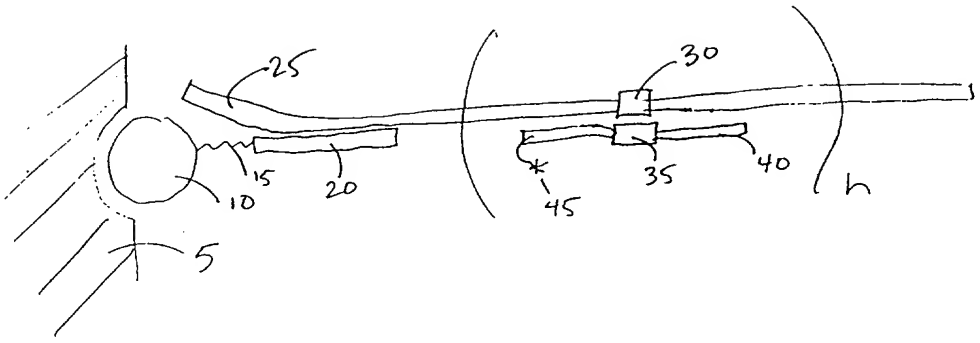


Figure 7 continued

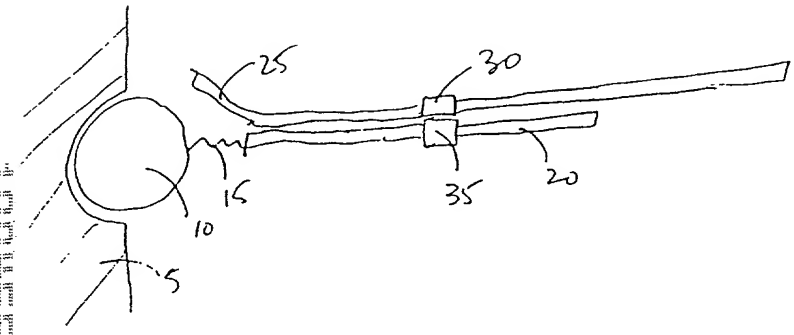


D

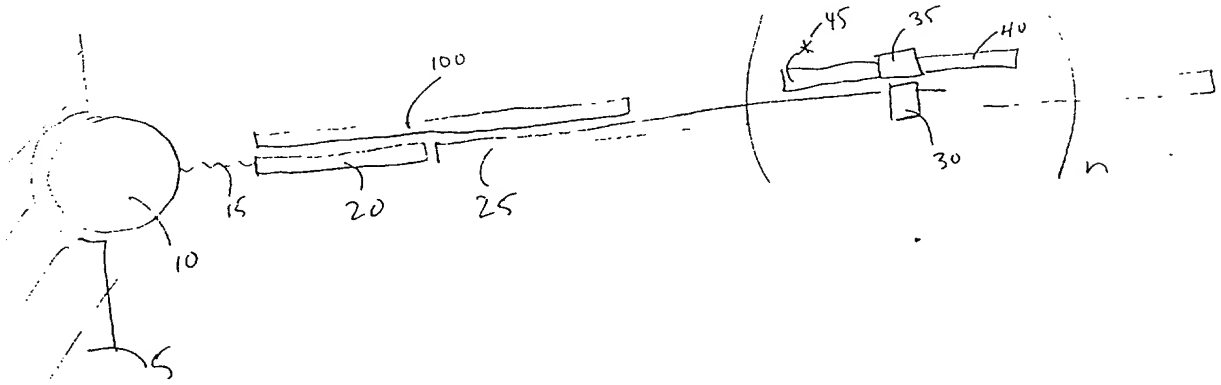
Figure 7 continued


25
30
15
20
10
35
40
45
n
5

A


25
30
15
35
20
10
5

B


45
35
40
100
30
n
15
20
25
10
5

C

FIGURE 8

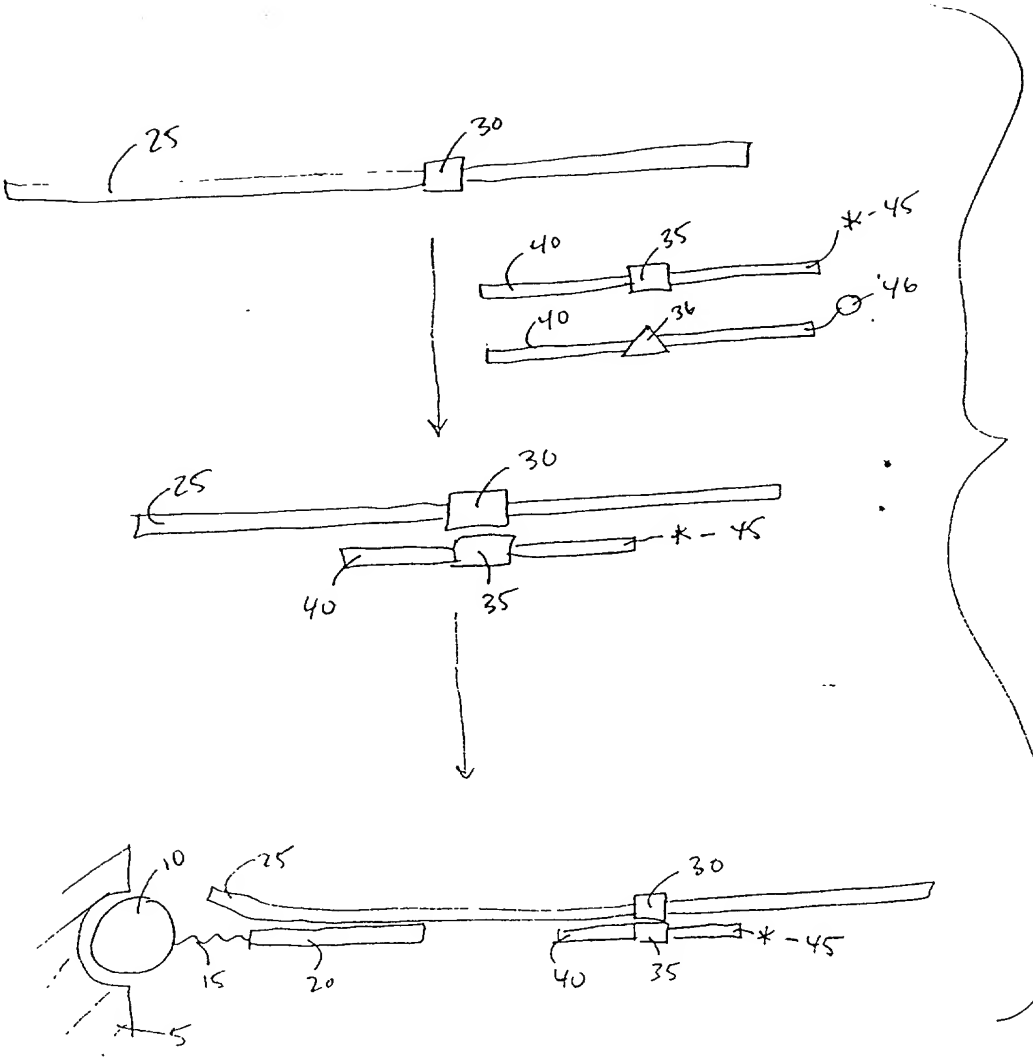
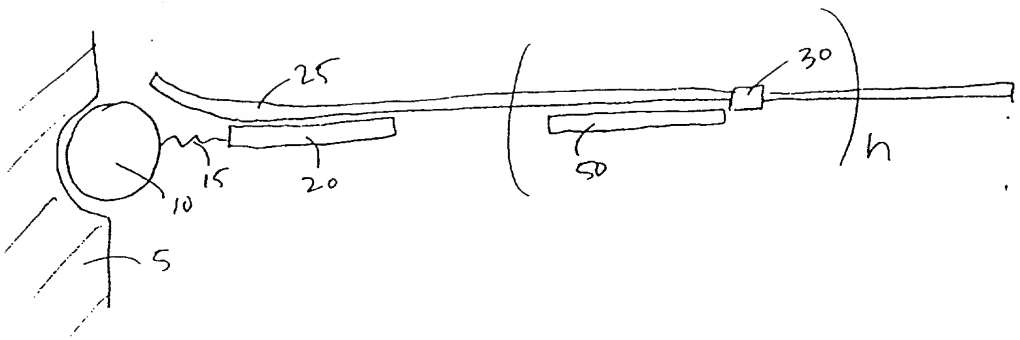
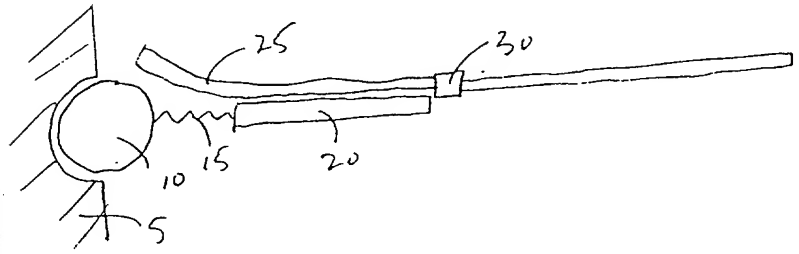


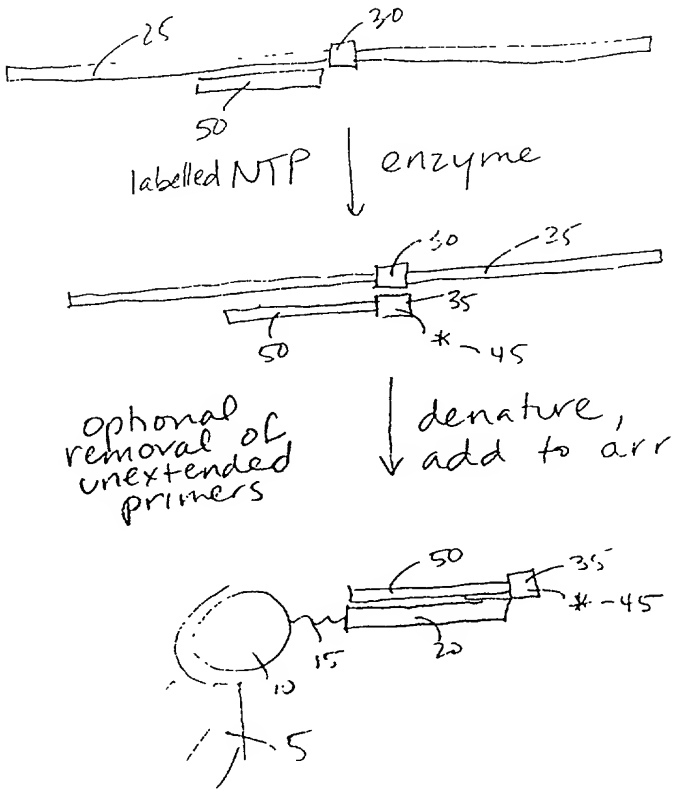
FIGURE 8
(continued)


25
30
15
20
50
10
5
h

A

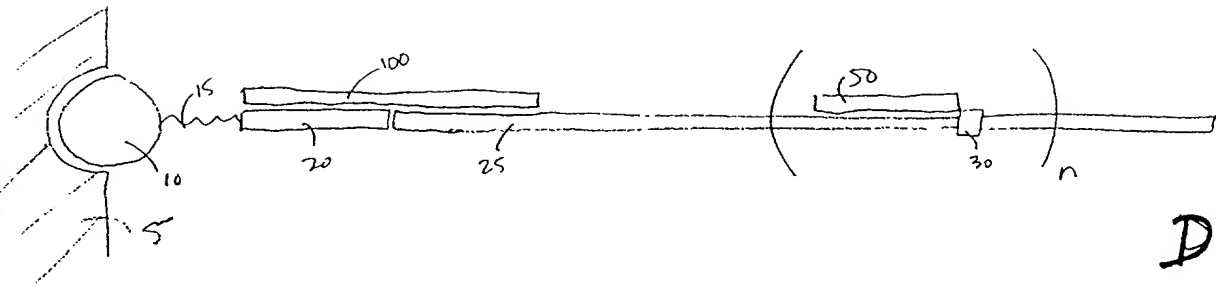

25
30
15
20
10
5

B


25
30
50
labelled NTP
enzyme
30
35
35
50
*-45
optional removal of unextended primers
denature, add to array
50
35'
*-45
15
20
10
5

C

FIGURE 9



D

FIGURE 9
(continued)

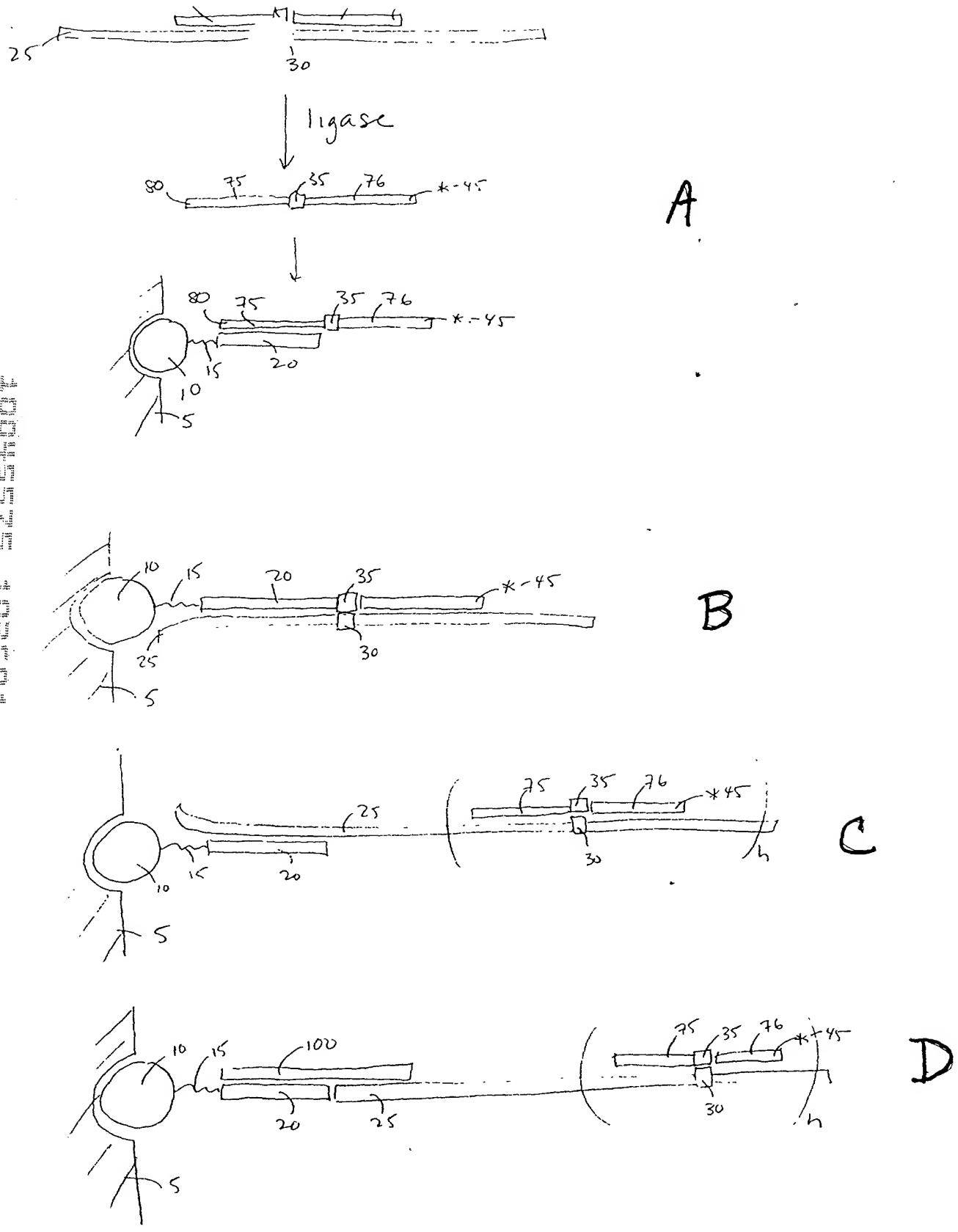
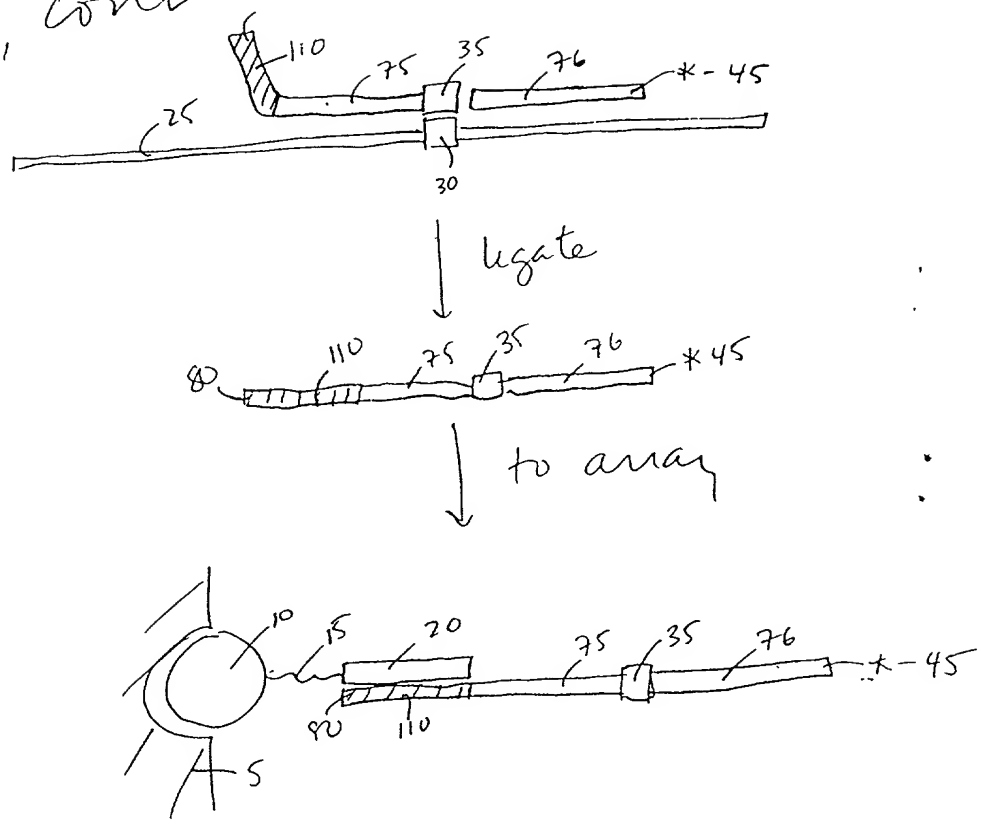

25
30
ligase
80
75
35
76
*-45
A
80
75
35
76
*-45
15
20
10
5
10
15
20
35
*-45
25
30
5
B
25
75
35
76
*45
30
h
10
15
20
5
C
10
15
100
20
25
75
35
76
*45
30
h
5
D

FIGURE 10

3. cont



E

FIGURE 10
(continued)

SPOLA Assay

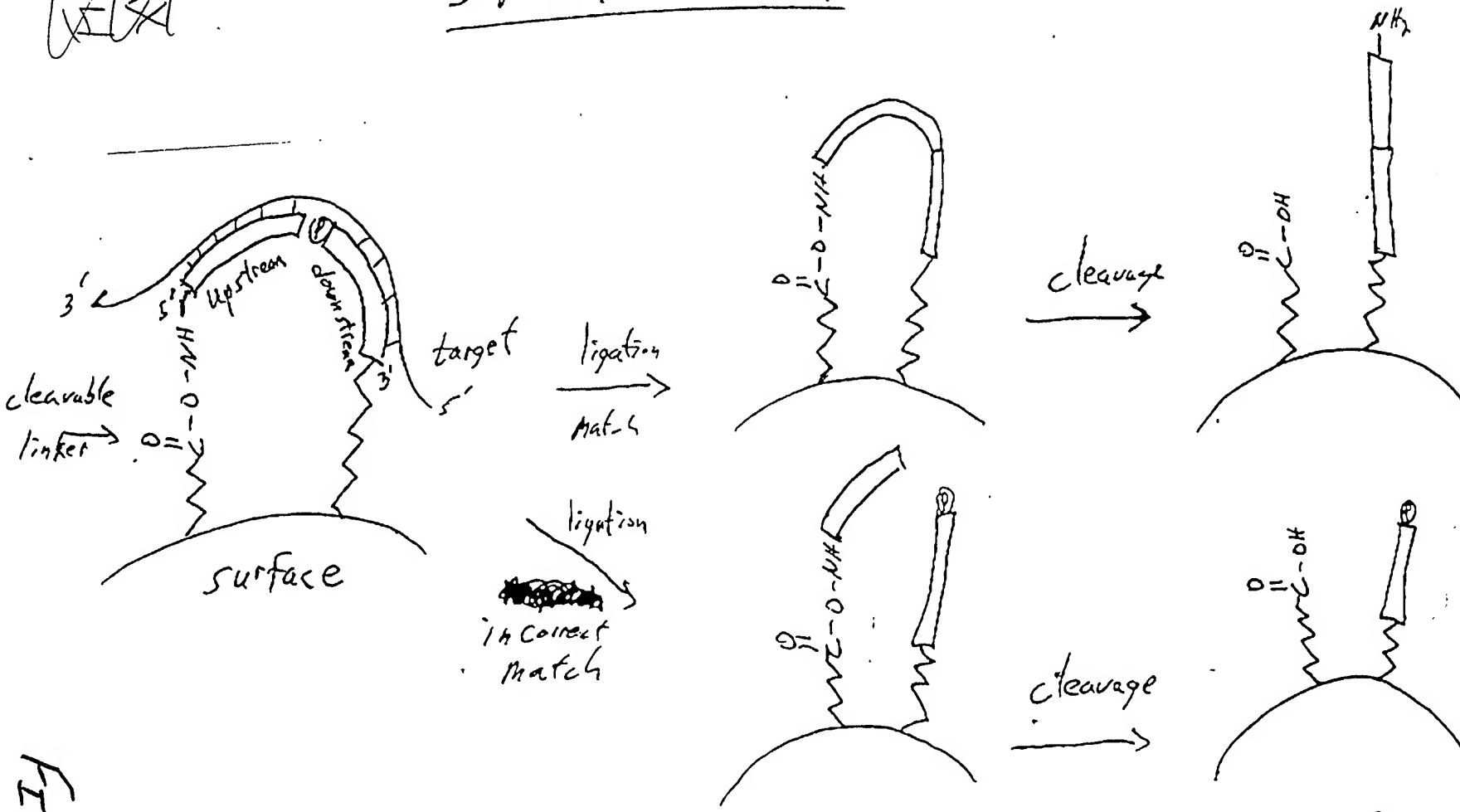


FIGURE 11

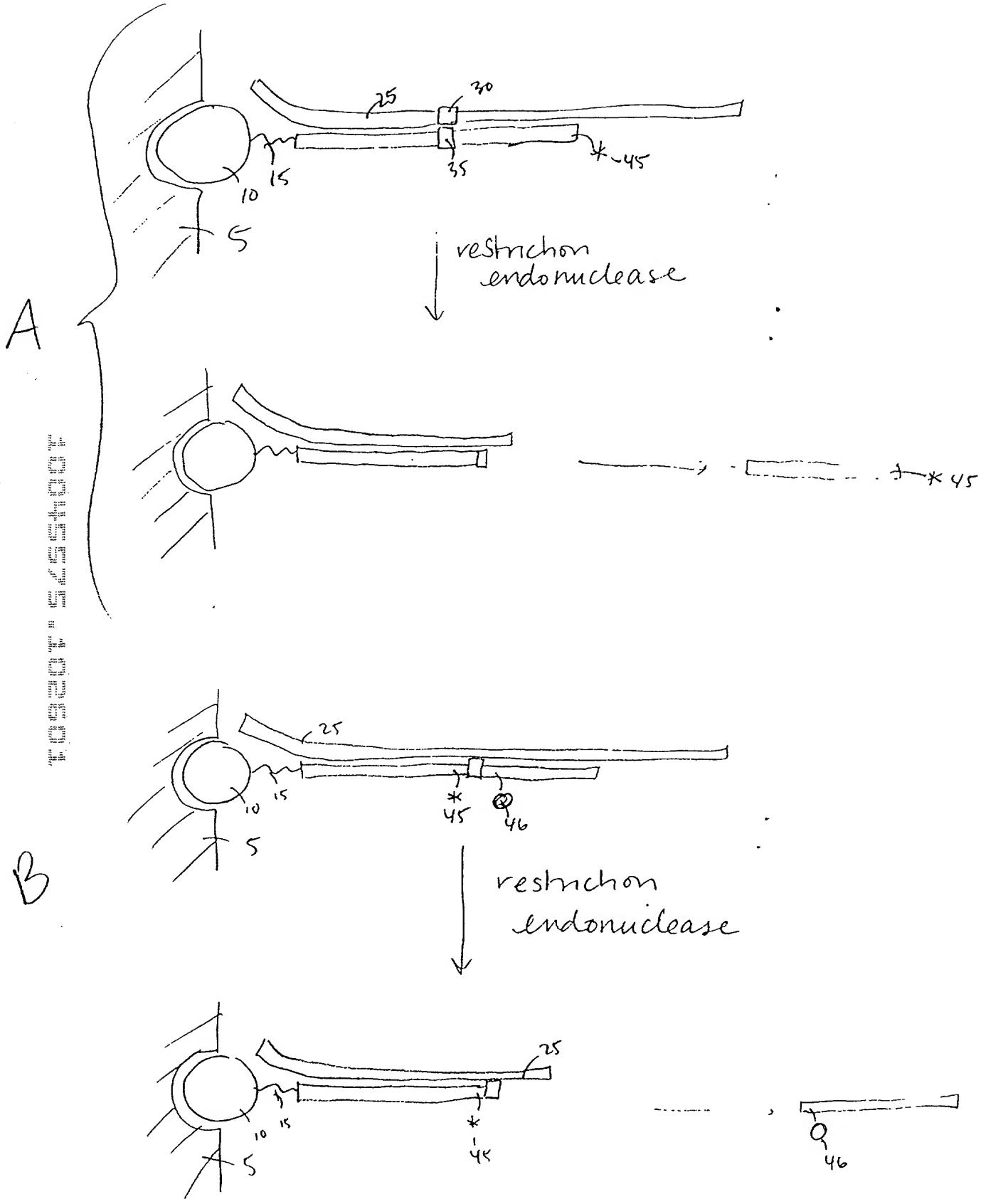
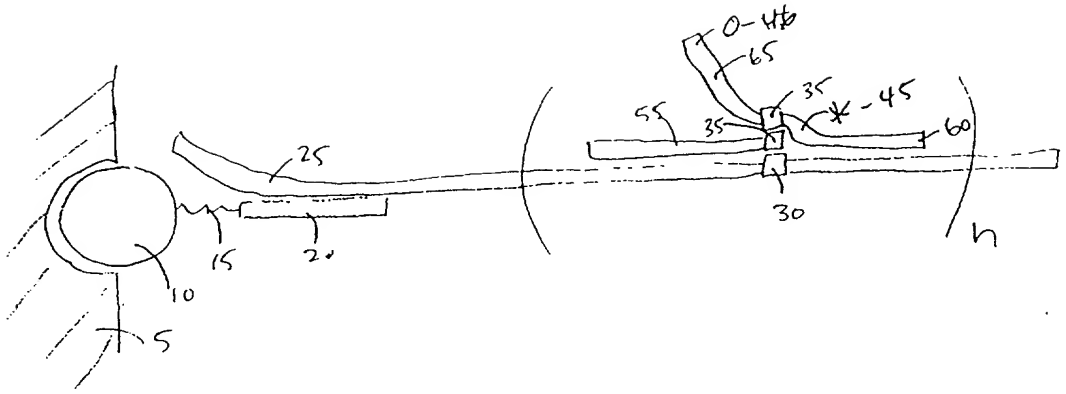
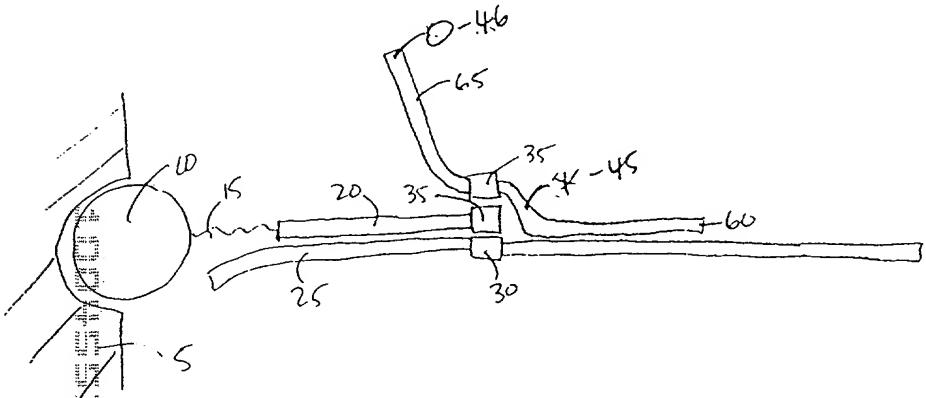

A
25
30
35
45
15
10
5
restriction endonuclease
45
B
25
15
10
5
45
46
restriction endonuclease
25
15
10
5
45
46

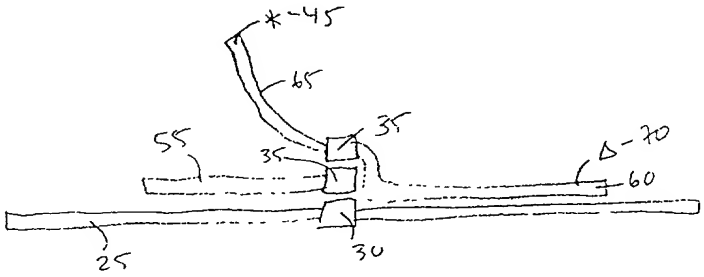
FIGURE 12



A

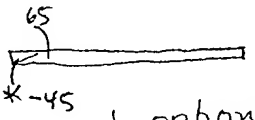


B



C

enzyme



ophonal remove uncleaved signal probe, add to array

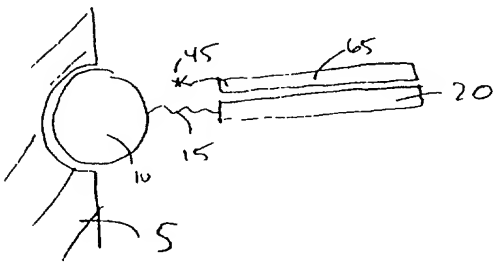
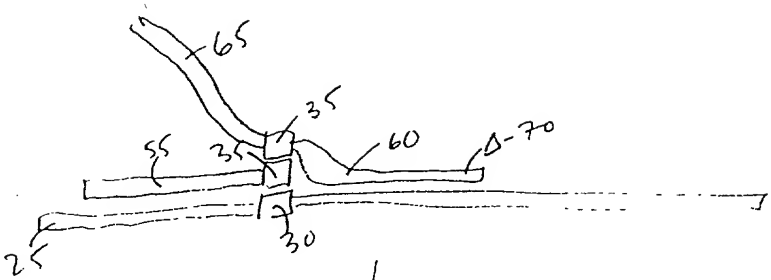
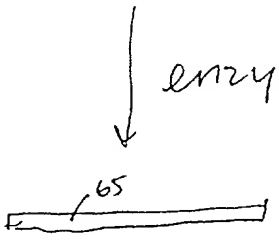
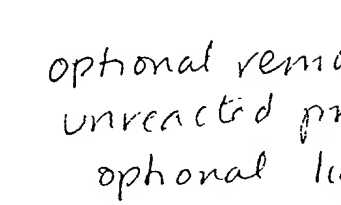


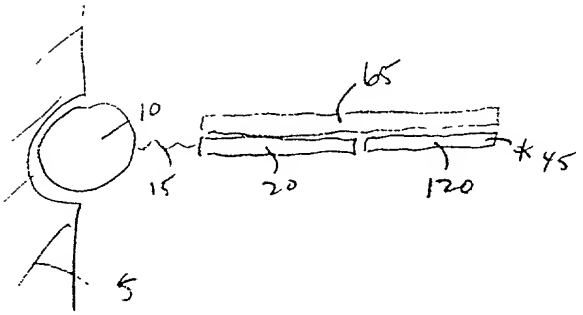
FIGURE 13

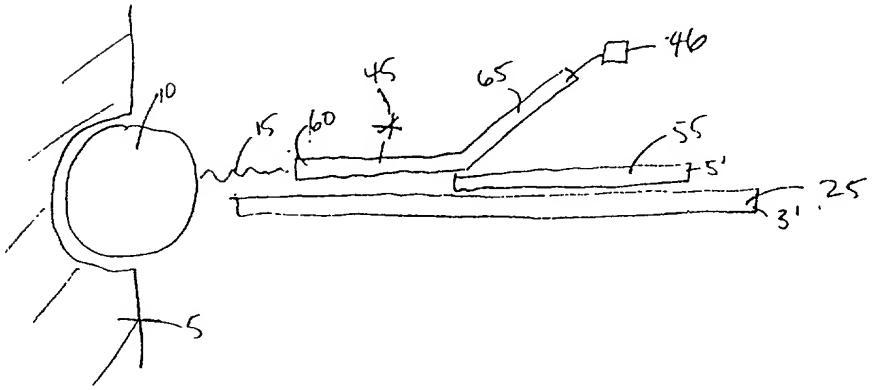




D







E

FIGURE 13
(continued)

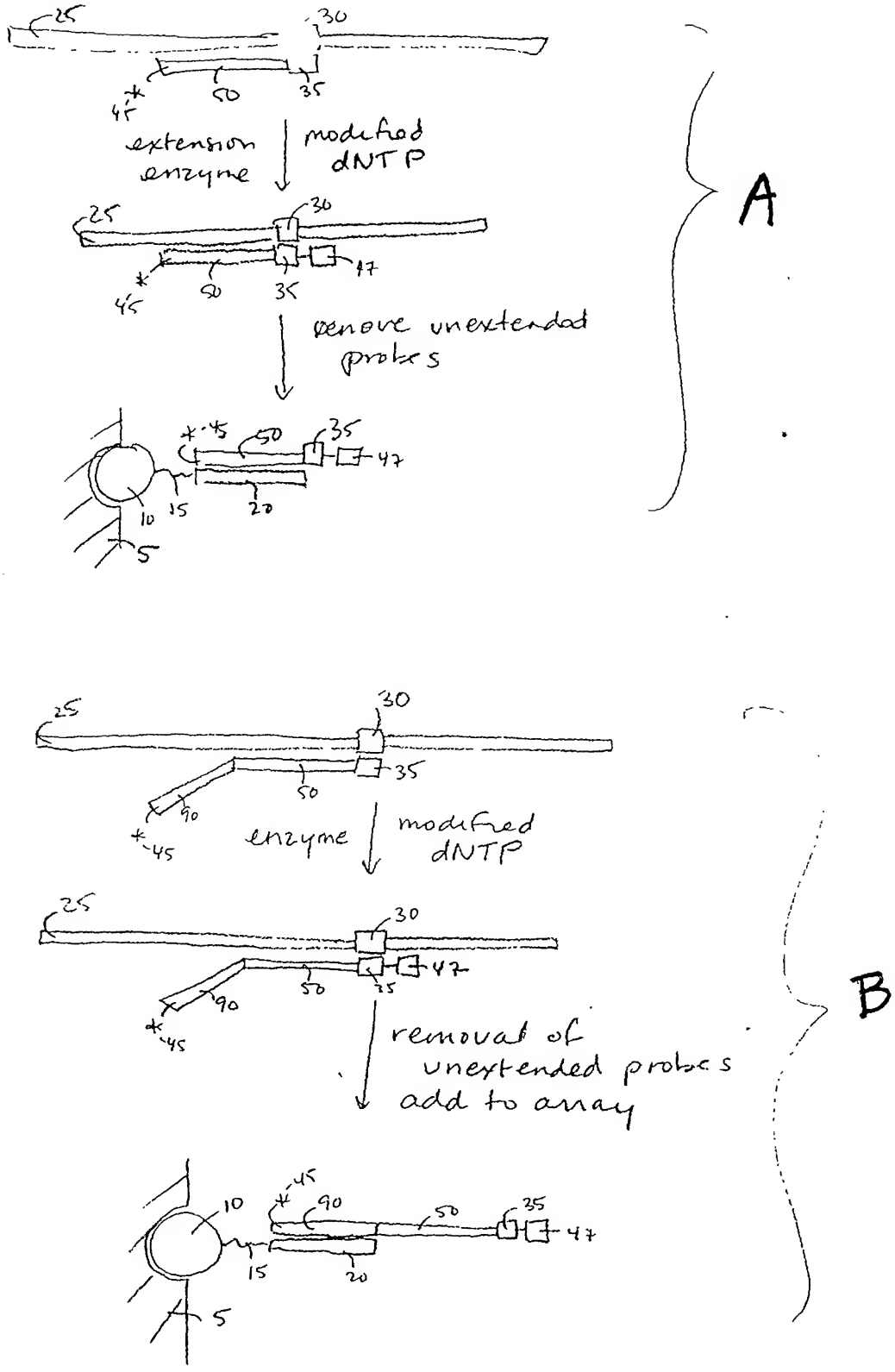

25
30
50
35
45
extension enzyme
modified dNTP
47
remove unextended probes
10
15
20
5
A
90
removal of unextended probes
add to array
B

FIGURE 14

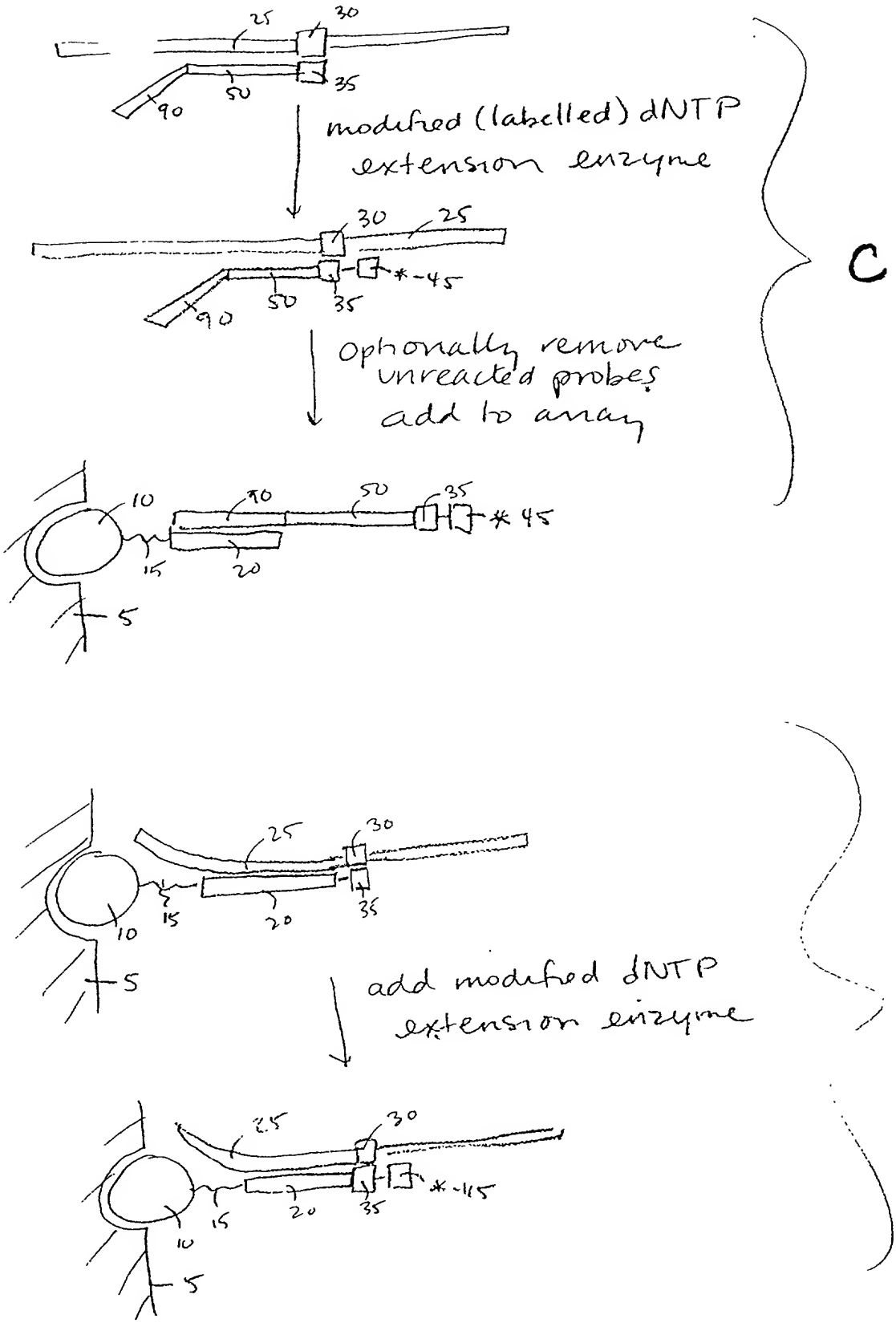


FIGURE 14 (continued)

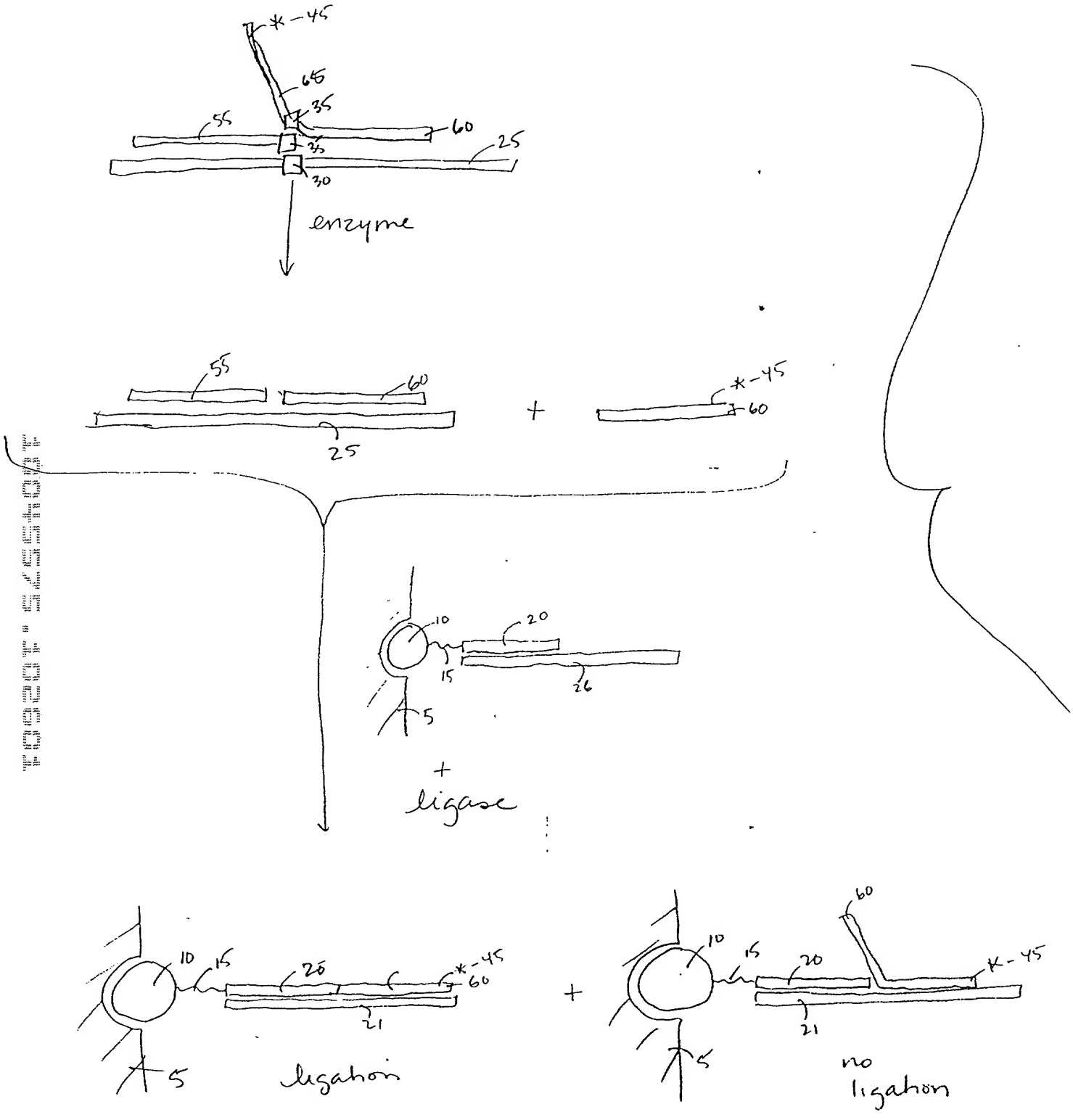
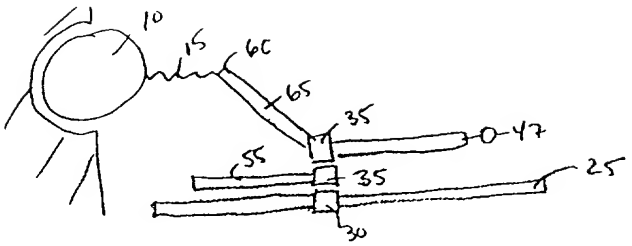
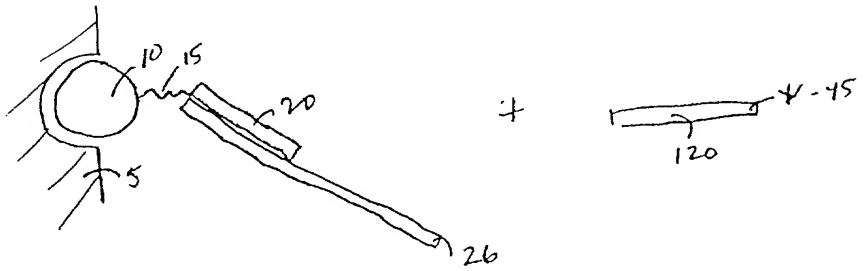

*-45
65
35
55
60
25
30
enzyme
55
60
25
+
*-45
60
10
20
15
26
5
+
ligase
10
15
20
*-45
60
21
5
ligation
+
60
10
15
20
*-45
21
5
no
ligation

FIGURE 15A



cleavage enzyme target template



ligation

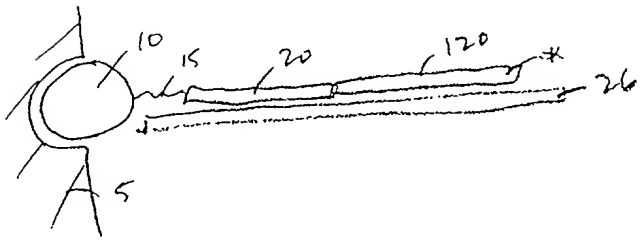


FIGURE 15B

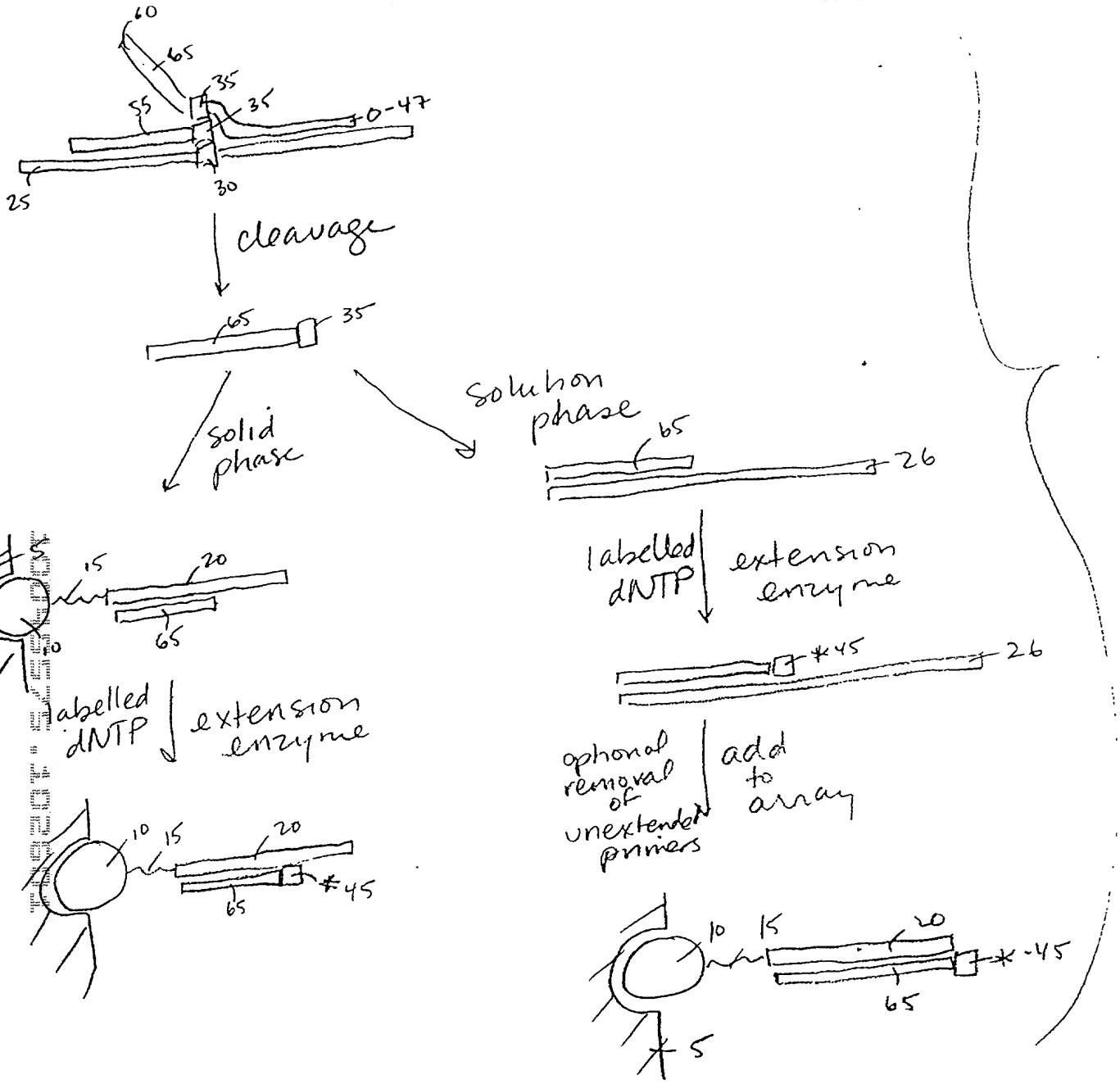

60
65
35
55
35
0-47
25
30
cleavage
65
35
solid phase
solution phase
65
26
5
15
20
10
65
labelled dNTP
extension enzyme
*45
26
optional removal of unextended primers
add to array
10
15
20
*45
65
5

FIGURE 16A

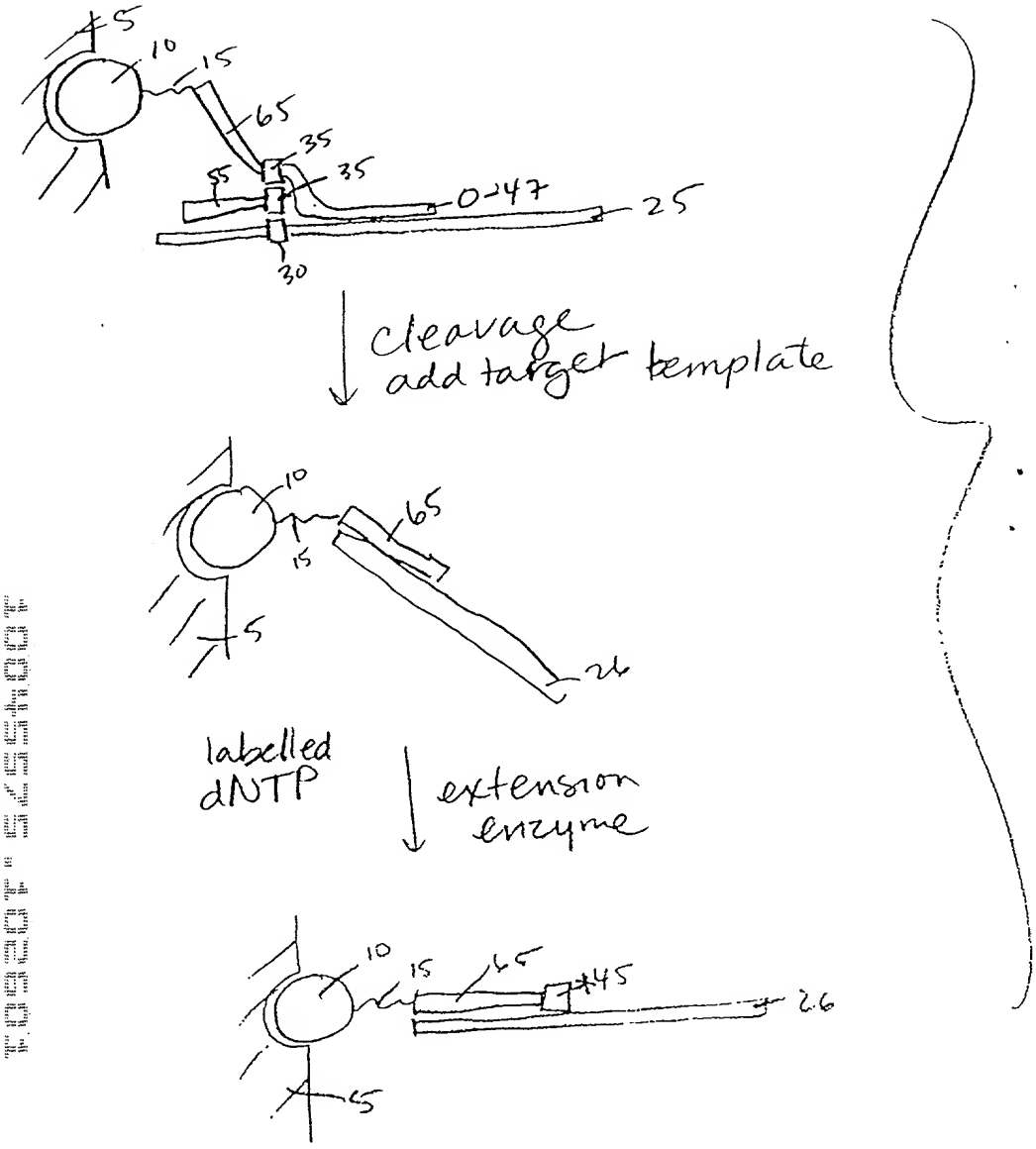
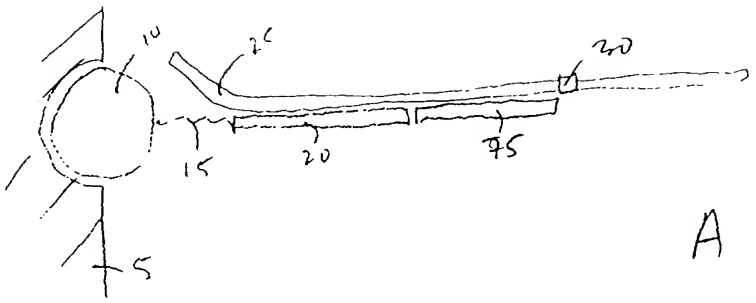
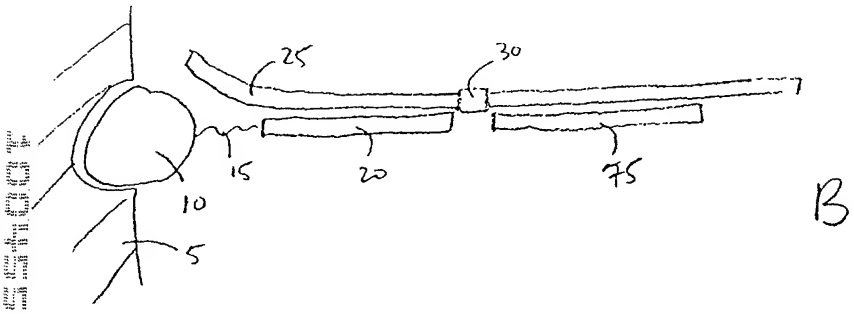

5
10
15
65
35
55
35
0-47
25
30
cleavage
add target template
10
65
15
5
21
labelled
dNTP
extension
enzyme
10
15
65
*45
26
5

FIGURE 16B

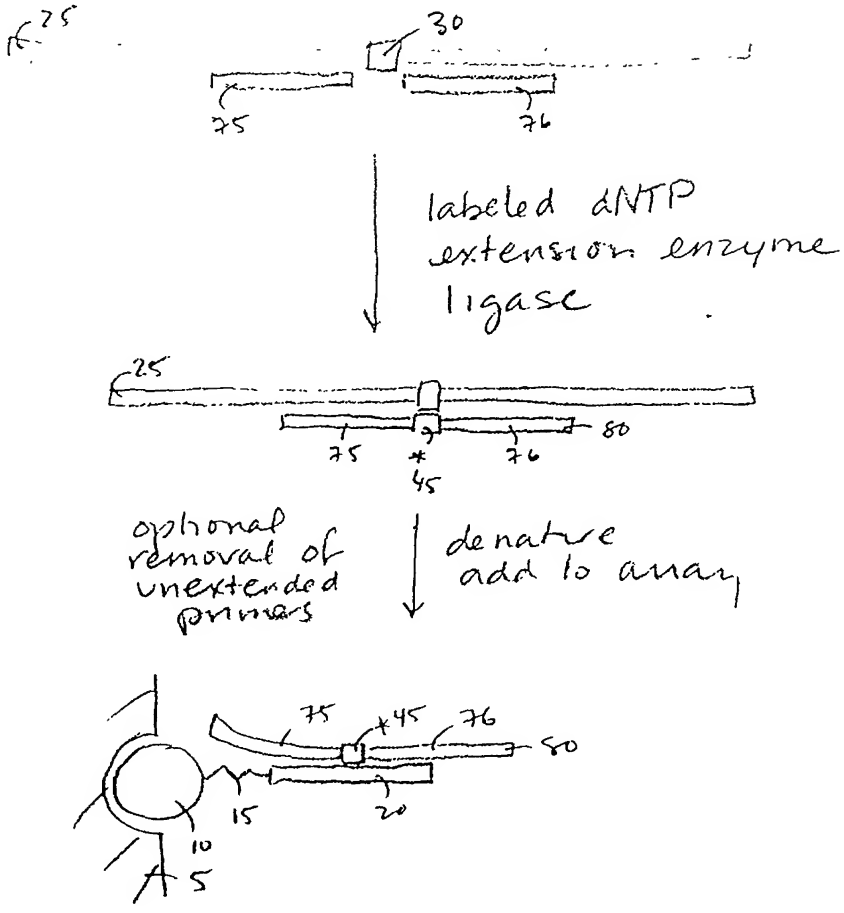

10
25
20
15
20
75
5

A

FIGURE 17


25
30
15
20
75
10
5

B


75
30
75
76
labeled dNTP
extension enzyme
ligase
25
80
75
*
45
76
optional
removal of
unextended
primers
denature
add to assay
75
*45
76
80
15
20
10
5

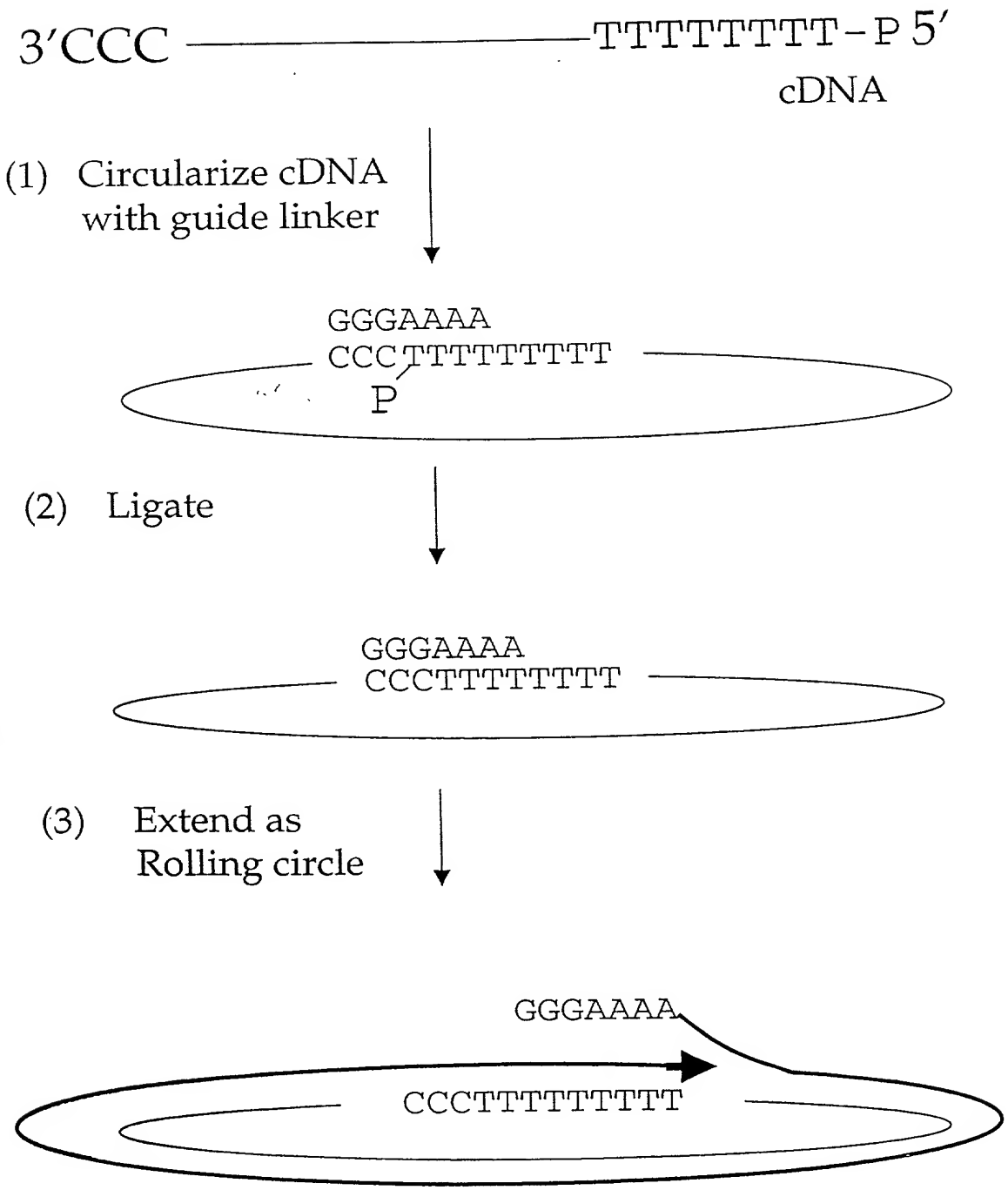

3′CCC
TTTTTTTT-P 5′
cDNA
(1) Circularize cDNA with guide linker
GGGAAAA
CCCTTTTTTTT
P
(2) Ligate
GGGAAAA
CCCTTTTTTTT
(3) Extend as Rolling circle
GGGAAAA
CCCTTTTTTTT

Figure 18

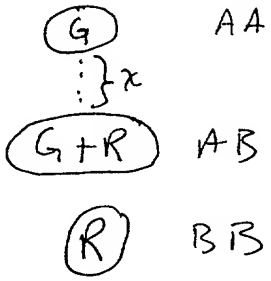
Single Labeled Probe

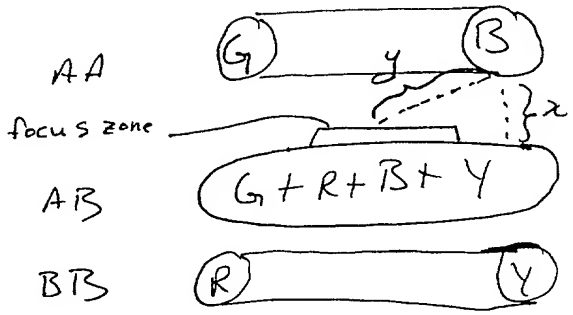
Genotype	Signal
AA	G/G
AB	G/R
BB	R/R

Multi-Labeled Probe

Genotype	Signal
AA	G, B / G, B
AB	G, B / R Y
BB	R, Y / R, Y

Signal Range





x = Single label distance
y = Multi label distance

Figure 19

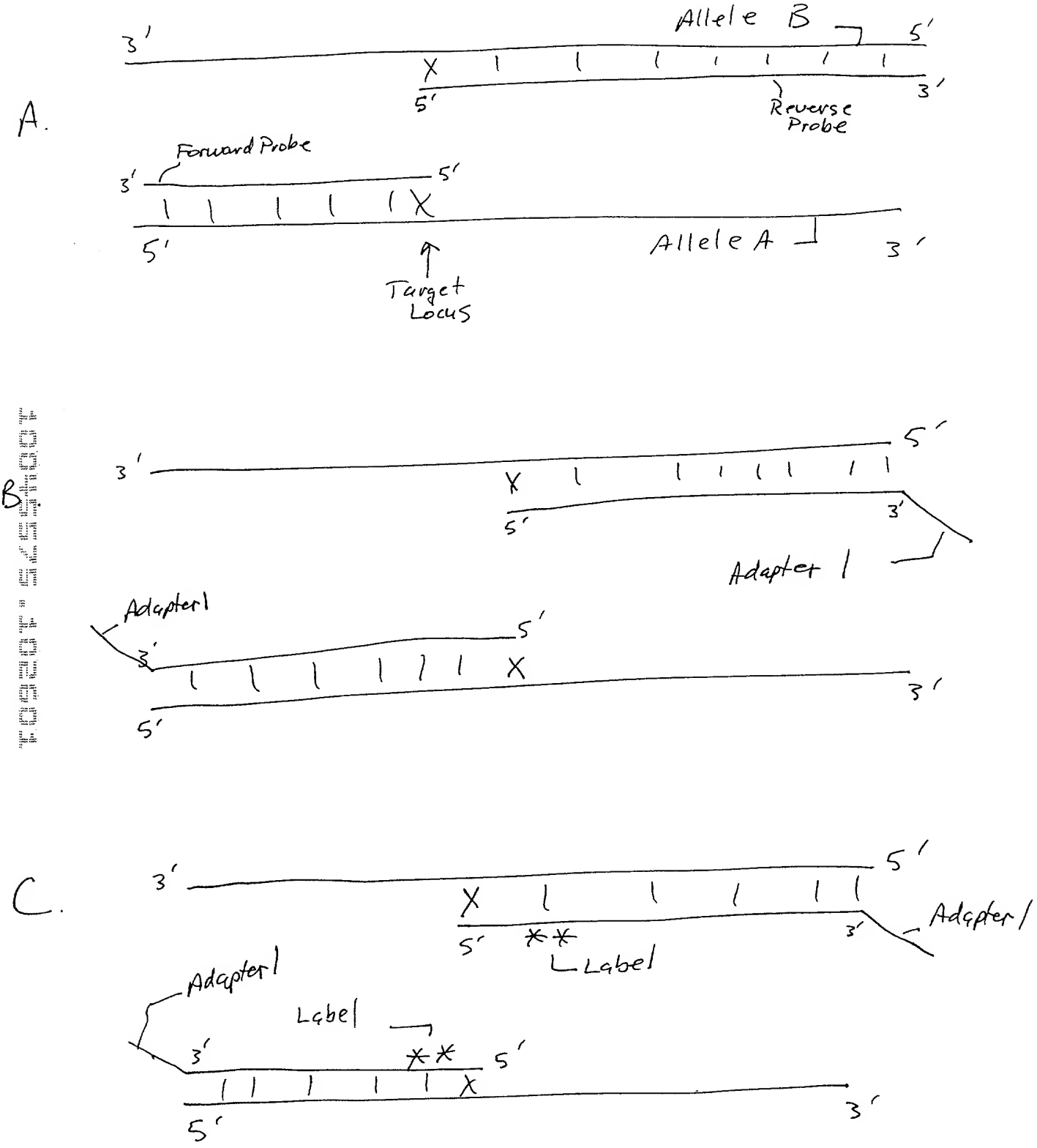

A.
Allele B
3'
5'
5'
3'
Reverse Probe
Forward Probe
3'
5'
5'
Allele A
3'
Target Locus
B.
3'
5'
5'
3'
Adapter 1
Adapter 1
5'
3'
5'
3'
C.
3'
5'
5'
3'
Adapter 1
Label
Adapter 1
Label
3'
5'
5'
3'

Figure 20

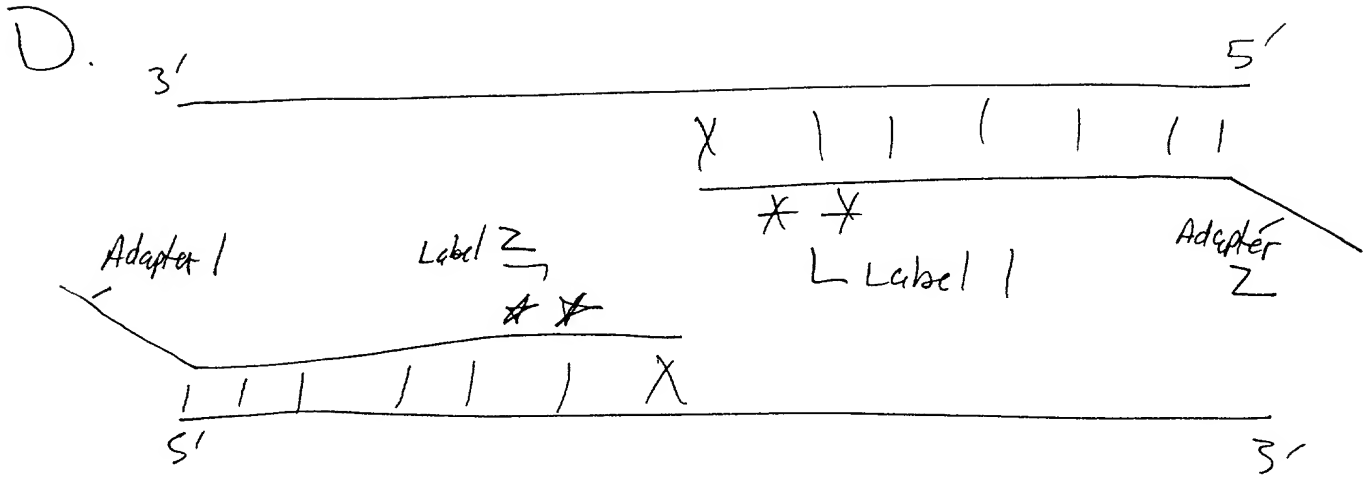


Figure 20 (continued)

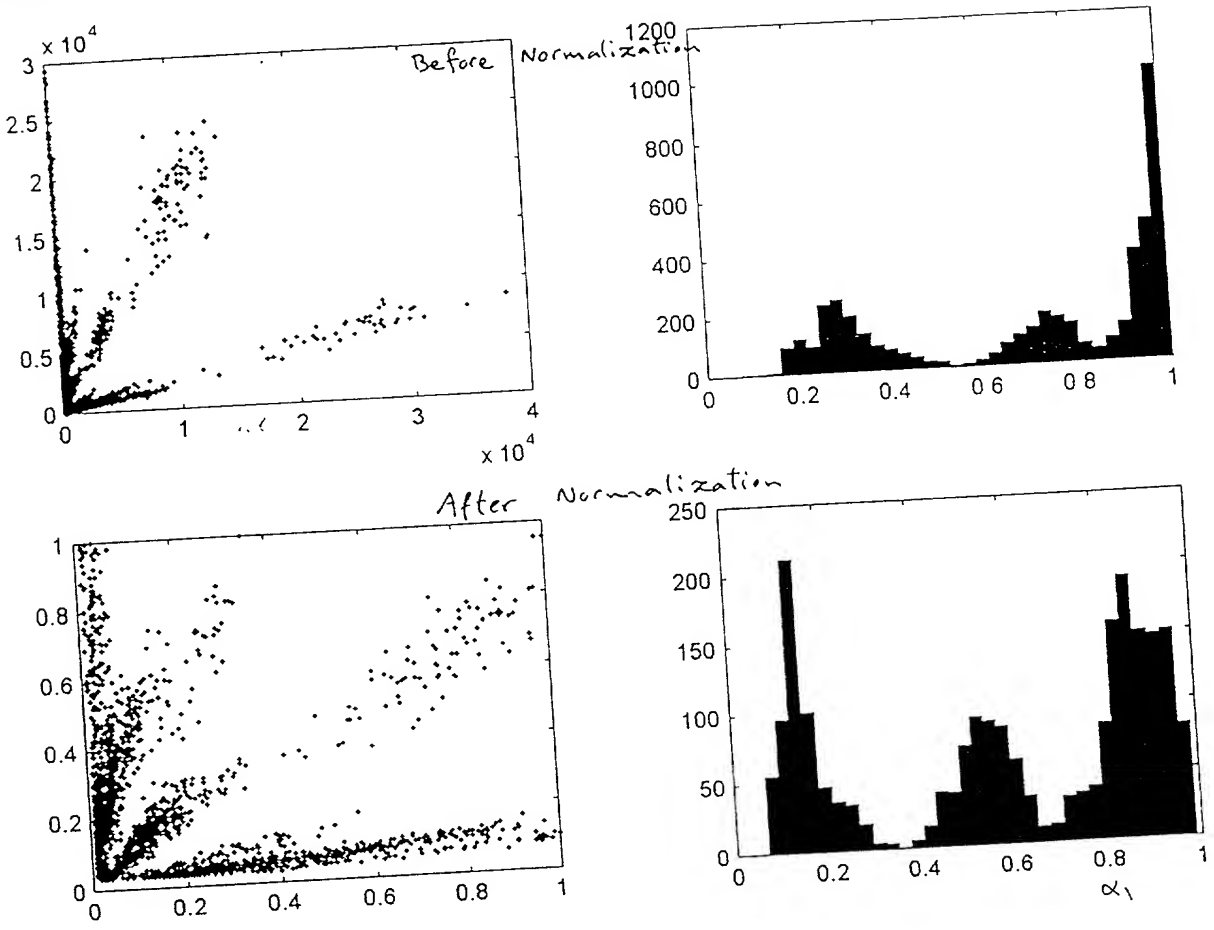
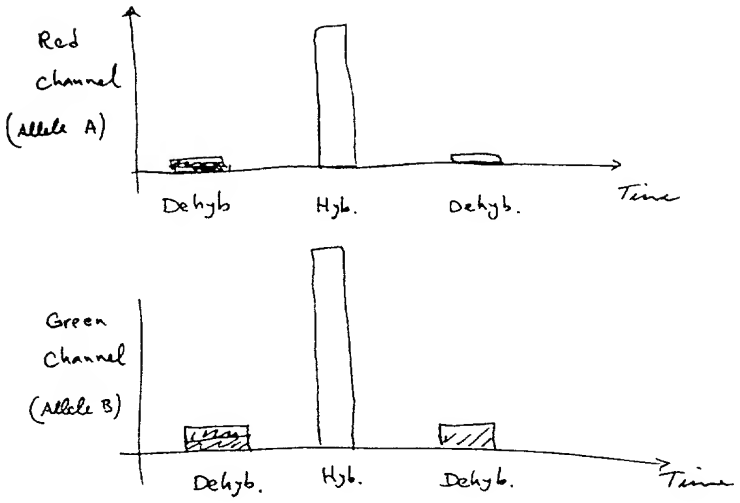
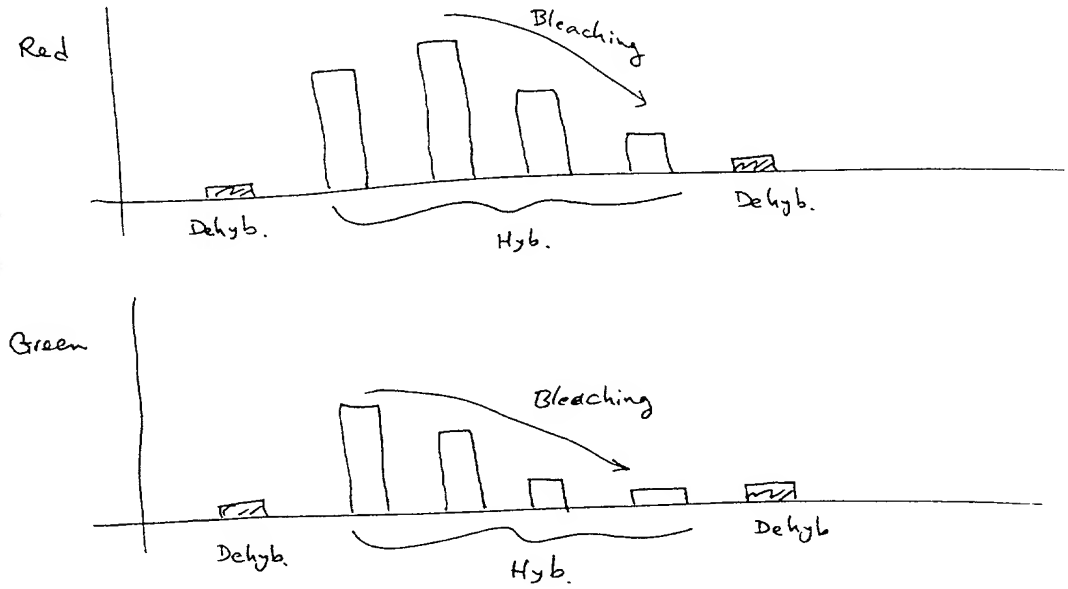

x 10^4
Before Normalization
x 10^4
After Normalization
α_1

Figure 21


Red
Channel
(Allele A)
Dehyb
Hyb.
Dehyb.
Time
Green
Channel
(Allele B)
Dehyb.
Hyb.
Dehyb.
Time

A


Red
Bleaching
Dehyb.
Hyb.
Dehyb.
Green
Bleaching
Dehyb.
Hyb.
Dehyb

B

Fig. 22

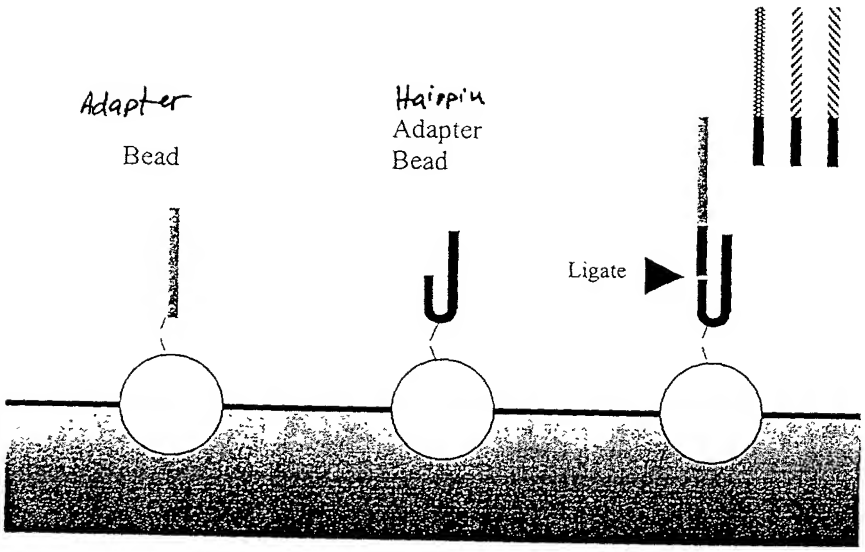
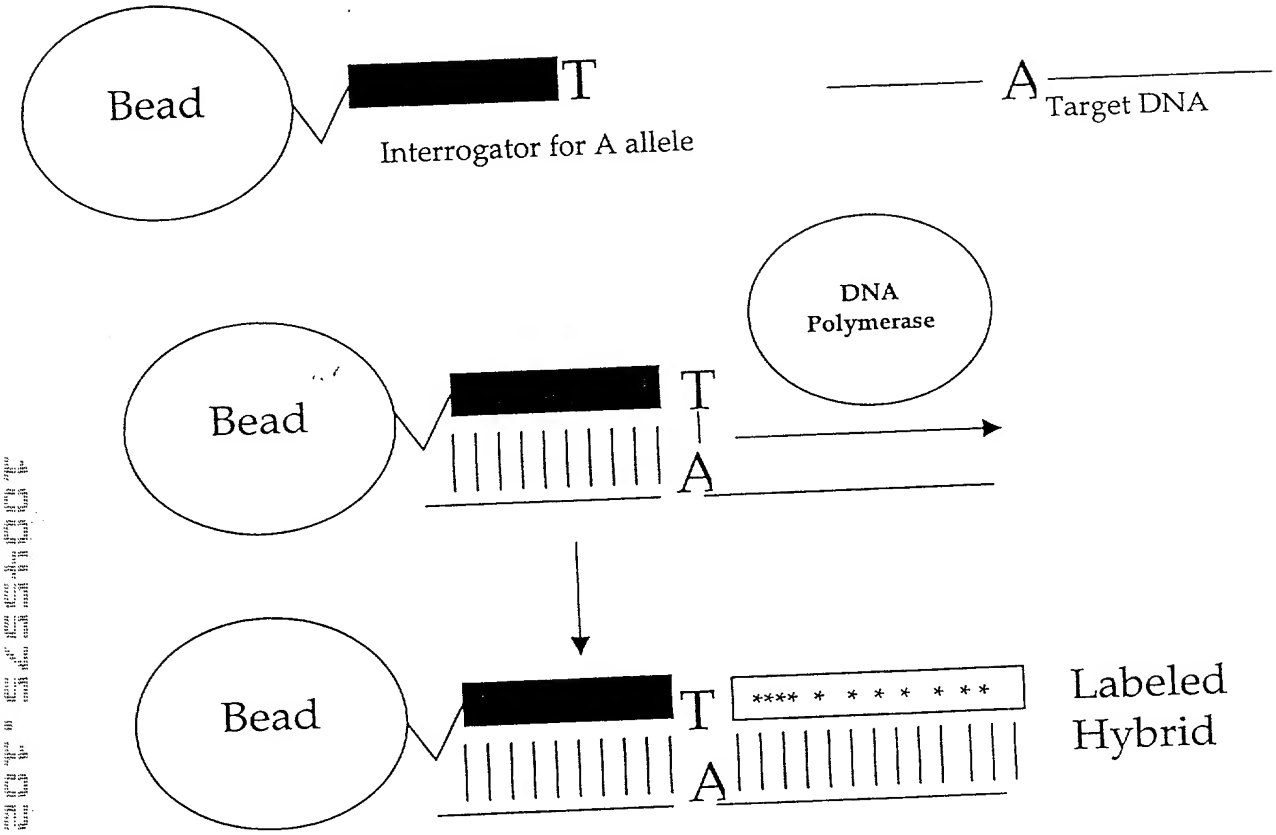


Figure 23

A. Match to SNP allele



B. Mismatch to SNP allele

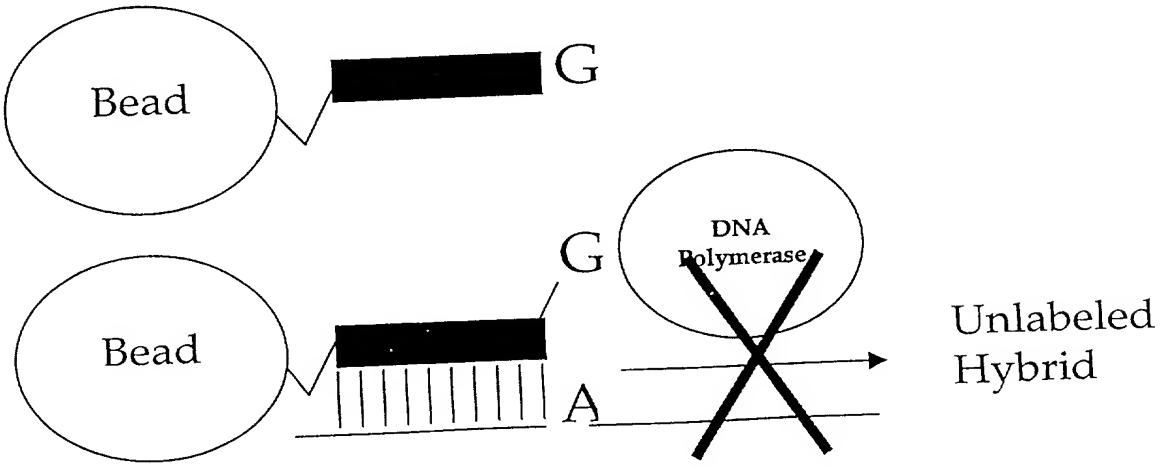
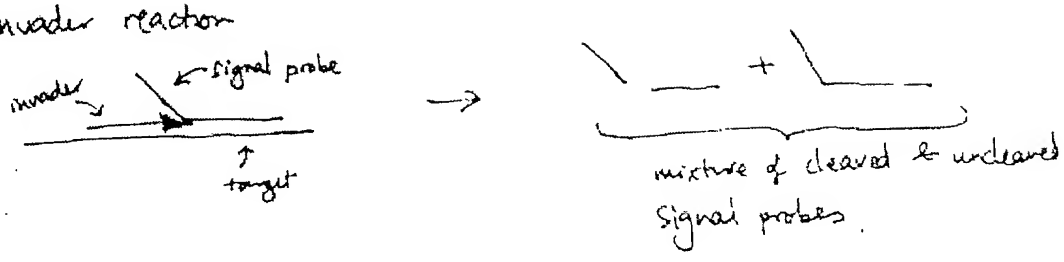


Figure 24

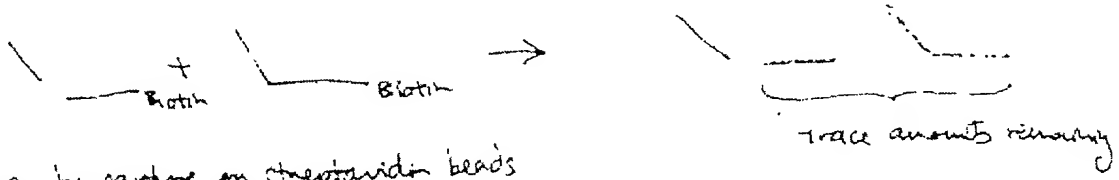
Invader - PCR

1

1) Invader reaction

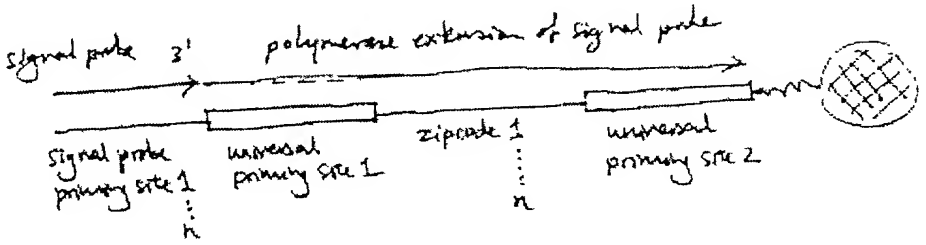


2) Removal of uncleaved signal probes



e.g. by capture on streptavidin beads
via 3'-biotin

3) Signal probe primes synthesis of amplicon target strand



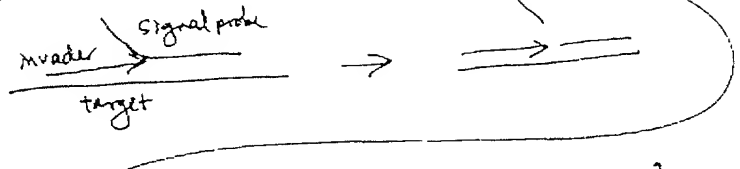
4) PCR amplification
newly synthesized target strands are denatured from template & transferred to PCR reaction (universal primers, dNTPs, taq polymerase) for multiplex PCR. Universal primers are labelled e.g. with biotin.

5) Array hybridization - PCR amplicons containing zipcodes are hybridized to array

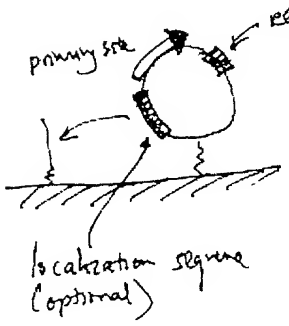
Figure 25

Invader - Rolling Circle

1) Invader reaction



Solid-phase version:



rolling circle template is tethered to surface e.g. to localized "features" in an array format, or to beads.

Rolling circle products can be localized e.g. by hybridization to adjacent probes or recovered in liquid phase for hybridization to a detection array. e.g. by enzymatic cleavage

Figure 26